# Subchondral Bone Abnormalities in Knee Osteoarthritis

By

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(Medical Research)

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# DECLARATION OF ORIGINALITY

The thesis contains no material which has been accepted for a degree or diploma by the University or any other institutions, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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# STATEMENT OF CO-AUTHORSHIP

This thesis includes papers for which Zhaohua Zhu (ZZ) was not the sole author. ZZ was the lead in the research of each manuscript; however, he was assisted by the co-authors, whose contributions are detailed below.

**Chapter 4:**

Zhu Z, Ding C, Jin X, Antony B, Han W, Laslett LL, Cicuttini F, Jones G. Patellofemoral bone marrow lesions: natural history and associations with pain and structure. Arthritis Care & Research. 2016 Nov; 68(11):1647-1654.

Author contributions:

ZZ collected the data, carried out analysis and interpretation of data, prepared the manuscript and completed manuscript revision. CD, GJ and FC participated in designing, analysing, interpreting and critically revising the paper. XJ, BA, WH and LL participated in data collection, manuscript drafting and revision, and data interpretation.

**Chapter 5:**

Zhu Z, Jin X, Wang B, Wluka A, Antony B, Laslett LL, Winzenberg T, Cicuttini F, Jones G, Ding C. Cross-sectional and longitudinal association between serum levels of high-sensitivity C-reactive protein, knee bone marrow lesions, and knee pain in patients with knee osteoarthritis. Arthritis Care & Research. 2016 Oct; 68 (10):1471-7.

Author contributions:

CD, GJ and FC designed the study. ZZ, WB and JX performed data management, collection and cleaning. ZZ, CD, GJ, BA, LL, TW and FC were involved in data analysis. All authors participated in manuscript revision and data interpretation. All authors approved the final version for publication.

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Author contributions:

ZZ, XJ, BW, WH and XW collected the data and involved in manuscript drafting. ZZ, CD, GJ, FC and TW designed the study and generated the hypothesis. ZZ, BA, PO and CD participated in data analysis and manuscript drafting. All authors were involved in interpreting the data, critically revising the manuscript and approved the final version for publication.

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Author contributions:

CD, GJ, and FC designed and carried out the study planning. CD and GJ participated in data analysis. ZZ, LL, XJ and ML collected the data, ZZ, LL and XJ involved in data analysis. ZZ, XW, WH and BA contributed to initial manuscript draft and preparation. All authors were involved in interpreting the data, critically revising the manuscript and approved the final version for publication.

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adults: a population-based cohort study. Osteoarthritis Cartilage. 2017 Dec; 25(12):2055-2062.

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CD, GJ and FC designed the study. ZZ, CD and GJ generated the study hypothesis and carried out the data analysis. ZZ, WH, and ML participated in data collection. ZZ, XJ, LL and BA involved in data analysis. ZZ, LL, XJ, WH and BA contributed to manuscript preparation. All authors were involved in interpreting the data, critically revising the manuscript and approved the final version for publication.

# STATEMENT OF ETHICAL CONDUCT

The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

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# PUBLICATIONS ARISING FROM THE THESIS

## Chapter 4

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adults: a population-based cohort study. Osteoarthritis Cartilage. 2017 Dec; 25(12):2055-2062.

# OTHER PUBLICATIONS

Zhu ZH, Jin XZ, Zhang W, Chen M, Ye DQ, Zhai Y, Dong FL, Shen CL, Ding C. Association between vitamin D receptor gene polymorphisms and osteoarthritis: an updated meta-analysis. Rheumatology (Oxford). 2014 Jun; 53(6):998-1008.

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Jin X, Wang BH, Wang X, Antony B, Zhu Z, Han W, Cicuttini F, Wluka AE, Winzenberg T, Blizzard L, Jones G, Ding C. Associations between endogenous sex hormones and MRI structural changes in patients with symptomatic knee osteoarthritis. Osteoarthritis Cartilage. 2017 Jul; 25(7):1100-1106.

Wang X, Cicuttini F, Jin X, Wluka AE, Han W, Zhu Z, Blizzard L, Antony B, Winzenberg T, Jones G, Ding C. Knee effusion-synovitis volume measurement and effects of vitamin D supplementation in patients with knee osteoarthritis. Osteoarthritis Cartilage. 2017 Aug; 25(8):1304-1312.

# SCIENTIFIC PRESENTATION AND AWARDS ARISING FROM THE THESIS

**2017**

*June*

The European League Against Rheumatism (EULAR) 2017 Annual European Congress of Rheumatology, Madrid, Spain

"Early osteophytes detected by MRI are associated with changes in knee pain and structures in older adults: a population-based cohort study" – *oral presentation* (Won **Travel Bursary**)

"MRI-detected knee osteophyte: natural history and structural risk factors affecting change" –*poster presentation*

"Interactions between steps per day and risk factors for osteoarthritis on MRI-detected osteophytes in a population based cohort study" – *poster presentation*

*April*

OARSI (osteoarthritis research society international) 2017 World Congress on Osteoarthritis, Las Vegas, USA

"MRI-detected osteophytes on knee: natural history and structural risk factors affecting change" –*poster presentation*

"Interactions between physical activity and risk factors of osteoarthritis on MRI-detected osteophytes in a population based cohort study" –*poster presentation & Breakfast Poster Tour*

**2016**

*November*

The 15th Australia National Conference of Emerging Researchers in Ageing, Canberra, Australia

"Standard x-ray undetectable osteophytes are associated with changes in knee pain and structures in older adults" -*oral presentation* (Won **Travel Bursary**)

"Association between MRI-detected osteophytes and changes in knee structures and pain" –*poster presentation*

ACR (American College of Rheumatology) Annual Meeting, Washington, DC. USA

"Association between MRI-detected osteophytes and changes in knee pain and structures in older adults"-*poster presentation*

*October*

The 23rd Australia Epidemiological Association Annual Scientific Meeting, Canberra, Australia

"Association between MRI-detected osteophytes and changes in knee structures and pain"-oral presentation.

"Standard x-ray undetectable osteophytes are associated with changes in knee pain and structures in older adults"-poster presentation (Won **Outstanding Presentation Award**)

*September*

The 37th SICOT (International Society of Orthopaedic Surgery and Traumatology) Orthopaedic World Congress, Rome, Italy.

"Cross-sectional and longitudinal associations between serum inflammatory cytokines and knee bone marrow lesions in patients with knee osteoarthritis"-*oral presentation*

"Pre-radiographic osteophytes are associated with changes in knee pain and structures in older adults"-*poster presentation*

APLAR (Asia Pacific League of Associations for Rheumatology) Congress, Shanghai, China

"Serum inflammatory cytokines and knee bone marrow lesions in patients with knee osteoarthritis"-*poster presentation*

**2015**

*September*

The 36th SICOT (International Society of Orthopaedic Surgery and Traumatology) Orthopaedic World Congress, Guangzhou, China.

"Associations between serum levels of hs-CRP, knee bone marrow lesions and knee pain in patients with knee osteoarthritis"-*oral presentation*

*June*

EULAR (Annual European Congress of Rheumatology) Congress, Rome, Italy.

"Associations between serum levels of hs-CRP, knee bone marrow lesions and knee pain in patients with knee osteoarthritis"-*poster presentation* (Won **Travel Bursary**)

*May*

OARSI (osteoarthritis research society international) 2015 World Congress on Osteoarthritis, Seattle, USA

"Serum levels of hs-CRP, resistin and associations with bone marrow lesions in patients with knee osteoarthritis"- *oral presentation*

"Patellofemoral bone marrow lesions: natural history and associations with pain and structure"-*poster presentation*

*April*

The 10th International Congress on Autoimmunity, Leipzig, Germany

"Longitudinal associations between serum interleukins and knee bone marrow lesions in patients with knee osteoarthritis"-*E poster presentation*

**2014**

*October*

Osteoarthritis Summit, Sydney, Australia

"Patellofemoral bone marrow lesions: natural history and associations with pain and structure"-*poster presentation*

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# ABSTRACT

Osteoarthritis (OA) is the most common type of arthritis, with prevalence estimates expected to increase dramatically worldwide due to ageing and increasingly obese populations. The knee is the most commonly affected joint, leading to pain, loss of function and disability. Magnetic resonance imaging (MRI)-detected bone marrow lesions (BMLs) and osteophytes (OPs) are types of subchondral bone abnormalities whose aetiology and predictive value are uncertain. The aims of this thesis are to investigate associations between local cartilage morphology, systemic inflammatory cytokines and knee BMLs, and the predictive value of MRI-detected OPs on knee OA structural and symptomatic changes.

Two data sources were used in this thesis. The first was a population-based study of older adults aged 50-80 years (mean age: 62 years; 51% female). Participants were randomly selected from the electoral roll in Southern Tasmania (population 229, 000) using sex-stratified random sampling. Follow-up measurements were performed at about 2.6 years later and again for questionnaire data at about 5.0 years later. MRI scans of the right knees were conducted at baseline and first follow-up. Knee cartilage defects, cartilage volume, tibial bone area, BMLs, effusion synovitis, infrapatellar fat pad and OPs were measured or scored based on MRI images. A standing anteroposterior semi-flexed view right knee with 15° of fixed knee flexion was performed at baseline with joint space narrowing (JSN) and radiographic OPs scored according to the Osteoarthritis Research Society International (OARSI) atlas. Knee pain was assessed using the Western Ontario McMaster Osteoarthritis Index (WOMAC) at all phases.

The second was a randomized, multi-centre, placebo-controlled and double-blinded clinical trial that was designed to evaluate the effect of vitamin D supplementation on knee OA. Eligible participants were aged 50 to 79 years who had symptomatic knee OA (according to American College of Rheumatology criteria) for at least 6 months, and had pain of 20 to 80 mm on a 100-mm visual analog scale (VAS). Additionally, participants' serum 25OHD levels were >12.5 nmol/L and < 60 nmol/L. Knee cartilage

defects, cartilage volume, BMLs and OPs were measured or scored based on MRI images at baseline and after 24 months. WOMAC knee pain, serum levels of inflammatory cytokines were assessed at baseline and after 24 months using enzyme-linked immunosorbent assay.

This thesis encompasses five studies. In the first study, the natural history of patellofemoral joint (PFJ) BMLs over 2.6 years was described and associations between PFJ BMLs, knee pain and knee cartilage morphology were evaluated in a population-based sample of older adults. 109 (27%) of 406 participants who completed follow-up had PFJ BMLs at baseline. Of these participants, 49 (45%) of these participants' PFJ BMLs persisted in the same grade, 26 (24%) increased in grade, and 34 (31%) decreased in grade. Change in PFJ BMLs over 2.6 years was deleteriously associated with change of knee pain when going up/down stairs over 5 years. Baseline PFJ cartilage morphology predicted increases in PFJ BMLs over 2.6 years.

In the second study, cross-sectional and longitudinal associations between serum high sensitivity C reactive protein (hs-CRP), knee BMLs and knee pain were investigated in a sample of knee OA patients. In these patients, serum hs-CRP is associated with knee BML scores and pain both cross-sectionally and longitudinally, suggesting inflammation is linked with BMLs and their associated pain.

The third study described cross-sectional and longitudinal associations between serum levels of IL-17A, IL-17F, IL-23, IL-6 and knee BMLs in patients with knee OA. Baseline IL-6 were significantly associated with total knee BMLs as well as increased knee BML scores in both females and males. Baseline IL-17F and IL-23 predicted increased BML scores in females only.

In the fourth study, MRI-detected OPs were measured and the cross-sectional and longitudinal association with knee structural abnormalities and knee pain were examined in older adults. Baseline MRI-detected OPs were significantly, independently and site-specifically associated with increases in cartilage defects, BMLs and loss of cartilage volume over 2.6 years. Medial tibiofemoral and total OP scores

were dose-dependently associated with total knee pain change over 2.6 and 5 years but these became non-significant after further adjustment for cartilage defects and BMLs.

In the fifth study, the prevalence of MRI-OPs detected only by MRI but not by standard x-ray was described, and the longitudinal associations with knee pain and structural changes were investigated in a population-based older adult sample. The prevalence of MRI-OPs was about 75%. Compared with participants without any OPs, participants with MRI-OPs had greater cartilage volume loss and increased cartilage defects and BMLs. MRI-OPs and established-OPs both predicted progression of knee structural abnormalities, but the associations for MRI-OPs were not as prominent as those for established-OPs. This suggests MRI-OPs may have a role to play in knee early-stage osteoarthritic progression.

In conclusion, this series of studies indicate that BMLs are not static and changes in BMLs are clinically relevant. Systemic inflammation is important in the aetiology of BMLs in OA. MRI-detected OPs can predict knee OA structural and symptomatic progressions. MRI-OP detected only by MRI but not by standard x-ray can also lead to OA progression, suggesting MRI-OP, which largely represent early OP formation, can also serve as a biomarker to predict knee structural progression over time.

# LIST OF ABBREVIATIONS

| | |
|---|---|
| 3D | Three-dimensional |
| 2D | Two-dimensional |
| 25OHD | 25-hydroxyvitamin D |
| ACR | American College of Rheumatology |
| APCs | Antigen presenting cells |
| AQoL | Assessment of Quality of Life |
| BMI | Body mass index |
| BML | Bone marrow lesion |
| BMD | Bone mineral density |
| Hs-CRP | High sensitive C-reactive protein |
| CI | Confidence interval |
| CV | Coefficient of variation |
| DXA | Dual-energy x-ray absorptiometry |
| DMOADs | Disease-modifying anti-OA drugs |
| EULAR | European League Against Rheumatism |
| ICC | Intra-class correlation coefficient |
| IL | Interleukin |
| JSN | Joint space narrowing |

| | |
|---|---|
| K/L | Kellgren and Lawrence |
| FSE | Fat-saturated T2-weighted fast spin echo |
| KOOS | Knee injury and Osteoarthritis Outcome Score |
| LDL | Low-density lipoprotein |
| MMP | Matrix metalloproteinase |
| MRI | Magnetic resonance imaging |
| HA | Hyaluronic Acid |
| MOST | Multi-Centre Osteoarthritis Study |
| COMP | Catilage oligomeric matrix protein |
| NHMRC | The National Health and Medical Research Council |
| NSAIDS | Non-steroidal anti-inflammatory drugs |
| OA | Osteoarthritis |
| OP | Osteophyte |
| OARSI | Osteoarthritis Research Society International |
| OR | Odds ratio |
| Pa | Per annum |
| PFJ | Patellofemoral joint |
| RA | Rheumatoid arthritis |
| RCT | Randomised controlled trial |
| ROA | Radiographic osteoarthritis |
| SD | Standard deviation |

| | |
|---|---|
| TASOAC | Tasmanian Older Adult Cohort Study |
| Th-17 | T-helper 17 cells |
| TKR | Total knee replacement |
| TKA | Total knee arthroplasty |
| VAS | Visual analog score |
| VDR | Vitamin D receptor |
| WOMAC | Western Ontario and McMaster Universities Osteoarthritis Index |
| VIDEO | Vitamin D Effect on Osteoarthritis Study |
| WORMS | Whole-Organ Magnetic Resonance Imaging Score |

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# Chapter 1: Introduction

## 1.1 Overview of osteoarthritis

### 1.1.1 What is osteoarthritis

Osteoarthritis (OA) is a progressive disorder of joints that results from breakdown of joint cartilage and subchondral bone [1]. OA is the most common form of arthritis and a leading cause of pain and disability among the elderly [2]. Although OA can damage any joint, the disorder most commonly affects knees, hips, spine and hands.

The disease manifests first as a molecular derangement, followed by anatomic, and/or physiologic derangements characterized by cartilage degradation, bone remodelling, osteophyte formation, joint synovities and joint function limitation [3]. The chronology of the OA trajectory from molecular disease to illness is still largely unknown, and it usually takes decades from clinical manifestations to organ failure when joint replacement is required [3].

OA is a progressive disease with many pathological changes in many structures, such as subchondral bone, cartilage, ligament, meniscus, periarticular muscle, synovium, nerves and periarticular fat [4]. It is considered highly heterogeneous in terms of sites, risk factors and syndromes, but eventually leads to common clinical manifestations [5, 6]. It is crucial to acknowledge that OA is far from a static disease. Several OA phenotyping systems have been proposed based on modern imaging [4], or pathophysiological mechanisms [5]. Those well-received subtypes include the following: metabolic OA [7], traumatic OA [8], atrophic/hypertrophic OA [9], inflammation-driven OA [10] and subchondral bone turnover- driven OA [11]. Developing personalized treatments targeting a particular disease phenotype with the etiologically appropriate therapy should result in far better therapeutic efficacy. Therefore, it is important to enhance our ability to identify factors that can more precisely classify various phenotypes of OA.

### 1.1.2 Osteoarthritis epidemiology

The prevalence of OA varies greatly depending on definitions used, specific joint, age group, gender and geographical area investigated. Generally, the incidence of knee, hip

and hand OA increases with advancing age, especially after age of 50 years, and females have higher risks than males [12]. A decreased incidence occurs around the age of 80 years possibly due to many people having the disease by that time [13]. In the Framingham Study, 19.2% radiographic knee OA was observed in adults aged ≥ 45 years, while in Johnston County Osteoarthritis Project it was 27.8% [14]. The third National Health Nutrition Examination Survey (NHANES III) estimated the prevalence of radiographic knee OA in the United States [15] was about 37% in adults aged >60 years. Murphy et al. reported the lifetime risk of developing symptomatic knee OA is about 40% in males, and 47% in females [16]. The prevalence of symptomatic knee OA was about 5% in Framingham participants aged ≥ 26 years, while it was 17% in participants aged ≥ 45 in the Johnston County Osteoarthritis Project [14]. In Australia, 9% of adults sefl-reported having OA, totalling 2.1 million Australians affected in 2014-2015 [17].

OA is the third-largest contributor to life-years lost due to disability in Australia [18]. OA has contributed to an estimated 60 000 disability-adjusted life-years (DALYs) lost in 2000 alone [19]. The Australian Institute of Health and Welfare (AIHW) reported that there are more than 40 000 new cases of radiological OA each year, which will continue grow as population ages [20], causeing a significant economic burden to society [18]. About $2.1 billion medical costs were attributed to OA from Australia healthcare budget in 2007 [21]. The leading component costs were hospitalisation, predominantly for joint replacement surgery (43%), clinic consultations (13%), medication (9%), and allied healthcare (6%). OA composes 63% of hospital inpatient expenditure, comparing to rheumatoid arthritis just 3.5% [22]. The group aged 45 and over, who is the most commonly affected by OA, is where most of the expenditure occurs, and the expenditure peaks in the 65-74 age group.

### 1.1.3 Diagnostic criteria

#### *Clinical*

The clinical diagnostic classification criteria developed by the American College of Rheumatology (ACR) remains a popular method for clinical and epidemiological

studies to diagnose OA [6]. Pain, stiffness, crepitus and Erythrocyte Sedimentation rate (ESR) are the key factors to consider in the diagnosis of OA using ACR clinical classification criteria, despite the fact that the natural history of OA most commonly begins long before any clinical symptoms are present.

Pain is usually the primary symptom of OA, with reduced range of motion and crepitus commonly present. Initially, pain may be relieved by rest or medications. But as OA progresses, pain may become more prominent and not respond to medications. The pain usually happens around the affected joint but in some cases, pain can also be referred to other areas. For instance, radiographic hip OA can manifest as knee pain [23].

Another common symptom of OA is stiffness. The morning stiffness of OA usually resolves within 30 minutes of rising, however, it can recur during the day through periods of inactivity [24]. Movement of OA joints may cause a crackling sound or grating sensation termed crepitus. Crepitus is presumably casused by roughening of the normally smooth joint surface [25]. ESR detects non-specific inflammation in the body. It is increased in rheumatoid arthritis (RA) but not in OA [24], and ESR < 40mm/hour is another ACR criteria. However, there is a growing consensus that low-grade systemic inflammation is related to OA development [26], but there are no consistent and reliable biomarkers thus far.

Recently, studies have argued that the ACR clinical criteria may reflect more advanced disease, and may be difficult to evaluate reliably [27-29]. There is an increasing awareness on the importance in identifying early or mild phases of OA degenerative process when there might still be some regenerative ability of cartilage and subchondral bone. This will allow to better design trials with increased benefit and reduced risks on disease-modifying anti-OA drugs (DMOADs) and lead to improved treatment algorithms in specific patient population.

***Radiographic***

Confirmation of the diagnosis can be made with radiography. Despite the development of advanced imaging techniques, conventional radiographs still are the gold standard for radiographic OA (ROA) diagnosis. The first criteria for ROA definition was

proposed in 1957 by Kellgren and Lawrence [30] and was embraced by the World Health Organization as standard criteria in 1961. The Kellgren and Lawrence (K/L) grading system has been extensively used in clinical research ever since. It offers an ordinal 5-point scoring scale to assess the severity of ROA based on the measurement of joint space narrowing (JSN) and osteophytes (OPs). Table 1 displays the K/L grading system in detail.

**Table 1** Criteria of Kellgren and Lawrence (K/L) grading system

| Grade – Changes | Description |
|---|---|
| **Grade 0- None** | No change |
| **Grade 1 –Doubtful** | Doubtful narrowing of joint space and possible osteophyte lipping |
| **Grade 2 – Minimal** | Definite osteophytes, definite narrowing of joint space |
| **Grade 3 – Moderate** | Moderate multiple osteophytes, definite narrowing of joint space, some sclerosis and possible deformity of bone contour |
| **Grade 4 - Severe** | Large osteophytes, marked narrowing of joint space, severe sclerosis |

Apart from the K/L grading system, alternative scoring systems that evaluate individual radiographic features have also been developed to screen patients for the diagnosis and staging of OA, particularly for clinical trials [31-33]. The Osteoarthritis Research Society International (OARSI) atlas classification score was established in 1996 [31], and later revised in 2006 [34]. Unlike the K/L grading system, the OARSI atlas uses semi-quantitative separate scoring for JSN and OPs (grading 0-3) in the medial tibiofemoral and lateral tibiofemoral compartment separately. The atlas has enabled standardisation of grading of OA from radiographs. Table 2 displays the OARSI atlas grading system details for the knee.

**Table 2** Criteria of OARSI atlas grading system for knee

| **Site & feature** | **Description** | | | |
|---|---|---|---|---|
| **Osteophytes** | | | | |
| *Medial femoral* | 0 (normal) | 1 (mild) | 2 (moderate) | 3 (severe) |
| *Medial tibial* | 0 (normal) | 1 (mild) | 2 (moderate) | 3 (severe) |
| *Lateral femoral* | 0 (normal) | 1 (mild) | 2 (moderate) | 3 (severe) |
| *Lateral tibial* | 0 (normal) | 1 (mild) | 2 (moderate) | 3 (severe) |
| **Joint space narrowing** | | | | |
| *Medial compartment* | 0 (normal) | 1 (mild) | 2 (moderate) | 3 (severe) |
| *Lateral compartment* | 0 (normal) | 1 (mild) | 2 (moderate) | 3 (severe) |
| Other | | | | |
| *Medial tibial attrition* | 0 (absent) | | 1 (present) | |
| *Medial tibial sclerosis* | 0 (absent) | | 1 (present) | |
| *Lateral femoral sclerosis* | 0 (absent) | | 1 (present) | |

Several additional grading systems are also available for assessing OA and its severity [35, 36].

Different scoring systems for ROA give markedly different prevalence estimates. For example, prevalence estimates for tibiofemoral OA using the OARSI atlas criteria are almost double than obtained using the K/L grading system [37]. This discrepancy may be a major reason for the variability of OA prevalence reported in the literature. However, the recent development of imaging technologies has revealed a number of inherent limitations of radiographs as an imaging tool for visualising OA [38], including poor sensitivity to follow-up changes; inability to delineate articular cartilage, meniscus, effusion synovitis, BMLs; effect of differences in knee positioning during imaging and lack of reproducibility [39].

### 1.1.4 Knee osteoarthritis

Knees are one of the joints most commonly affected by OA. Prevalence of symptomatic knee OA is 10% in men and 13% in women aged 60 years or older [40]. The prevalence of knee ROA (defined as KL grade 2 or higher) among elderly in United Stated is 30%-40% [41]. Risk factors for knee OA include previous injury, family history, female gender and obesity, but the most important risk factor is age. Knee OA is the most frequent cause of knee pain in adults aged > 50 years. Thus, knee OA needs to be taken seriously not only due to its high prevalence rate compared with other sites of OA but also for its occurrence at earlier age groups espacially in younger obese women [42].

### 1.1.5 PFJ osteoarthritis

The knee joint is usually considered as tri-compartmental: medial, lateral tibiofemoral and patellofemoral compartments. Traditionally, knee OA has been seen as a disorder of the tibiofemoral joint (TFJ) principally because radiographic assessment has focused on the anteroposterior X-ray, which does not image the patellofemoral joint (PFJ) [43]. As technological advances have enabled development of more sophisticated imaging tools, so too has awareness of PFJ OA. This is timely because PFJ OA is one of the most important source of pain and function limitation in knee OA [44-46]. Radiographic OPs in the PFJ are more common than the TFJ compartment in the community-based population [47]. The most common radiographic presentation in people with knee OA is combined TFJ and PFJ OA, followed by segregated PFJ OA, with segregated TFJ OA comprising only a very small proportion of participants (4% ) [48]. Despite the high prevalence and clinical relevance of PFJ OA, relatively little research has been done on this specific compartment, especially the natural history of OA features in this compartment has not been described.

### 1.1.6 Viamint D and osteoarthritis

Both vitamin D deficiency and OA are highly prevalent in the adult population of over 50 years of age [49]. The prevalence of OA and vitamin D deficiency increases with age and is higher in the obese people and in women [50]. A systematic review of 13 observational studies concluded that low levels of 25-(OH)D were associated with increased progression of radiographic knee OA. However, there was limited evidence

to support the association between 25-(OH)D levels and symptomatic OA [51]. Despite wide-ranging health benefit claims for vitamin D in OA, the randomised clinical trials (RCTs) conducted to explore the effect of vitamin D supplementation on OA have not found reduced knee pain or cartilage volume loss [52, 53]. As vitamin D insufficiency is common in OA populations, future research should address whether vitamin D supplementation over the long term is effective in OA patients with vitamin D deficiency.

## 1.2 Subchondral bone abnormalities

### 1.2.1 Preface

Subchondral bone abnormalities are primary players in the pathogenesis of OA [54]. Bone marrow oedema/lesions [55, 56], tibial bone size [57], bone attrition [58], bone mineral density [59], trabecular bone morphometry [60], bone cysts [61], bone shape [62] and osteophyte (OP) [63] are typical subchondral bone abnormalities that have been linked to OA symptoms and structural changes.

The subchondral bone has long been viewed as intimately associated with cartilage damage in the joint. Recent epidemiological studies with MRI have revealed that bone changes are evident in early disease stage, even prior to cartilage lesions [64]. This section summarizes the current knowledge of the role of two most well investigated bone changes in OA: BMLs and OP.

### 1.2.2 Bone marrow lesions

Bone marrow lesions (BMLs), previously known as bone marrow oedema, is described as ill-defined area of high signal intensity area in T2-weighted, fat-saturated MR images or in short tau inversion recovery (STIR) sequences. It usually cannot be visualized by x-ray, ultrasound or computed tomography (CT) [65, 66]. Histologically, BMLs consisted of a wide range of abnormalities such as abnormal trabeculae, bone marrow necrosis/remodelling, contusion and marrow fibrosis [67, 68]. In knee OA patients, the BML size fluctuates over time [69, 70], and the prevalence estimates have been found to vary from study to study [71, 72]. BMLs may decrease or increase in size in as little

as 6 weeks [73], resolve completely, or new BMLs may develop [74]. Figure 1.1 illustrates changes in BML size from time to time.

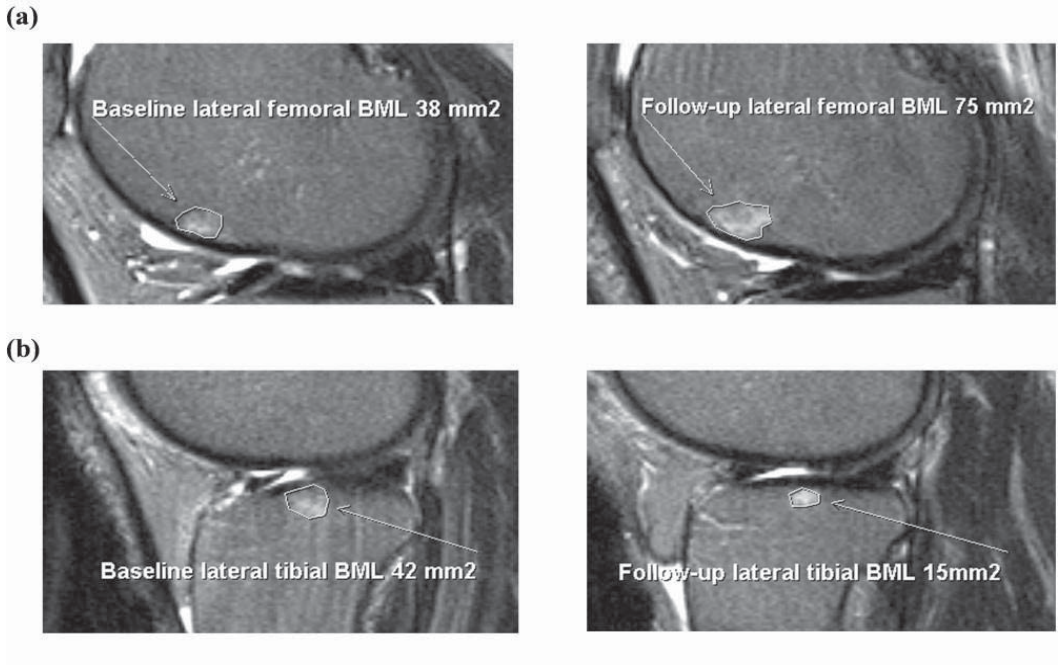



**Figure 1.1** Changes in BML size. a, BML increase from baseline to 2.7-year follow-up. b, BML decrease from baseline to 2.7-year follow-up

*Source: Dore et al. "Natural history and clinical significance of MRI-detected bone marrow lesions at the knee: a prospective study in community dwelling older adults." In: Arthritis Research & Therapy, 12.6 (2010),1186/ar3210.*

BMLs are strongly related to knee pain [75, 76], OA incidence [77] and progression [78]. Moreover, BMLs can predict knee JSN on x-ray [79], cartilage defect progression [55], cartilage volume loss on MRI [80] and total knee replacement [81]. Thus, BMLs are hallmarks of knee OA on MRI [65].

BMLs are associated with knee pain in most cohort studies [82], with estimated OR ranging from 2.0 [83] (adjusted for effusion and synovitis) to 5.0 (unadjusted) [84]. Hunter et al. [85] found that increased BML score was significantly associated with worsening of cartilage degradation. A cohort study suggested BML as a risk factor for rapid cartilage loss for both global knee and medial compartments [80]. The presence of BML predicts knee replacement independent of age, and patients with BMLs were

about 9 times more likely to have knee arthroplasty within 3 years compared with those without BMLs [86].

Despite associations with knee pain and structural changes, the aetiology of BML formation remains largely unknown. Suggestions include local inflammatory reactions to cartilage breakdown products [87]. However, evidence linking systematic inflammation and knee BMLs is lacking, especially in knee OA patients.

### 1.2.3 Osteophytes

Osteophytes (OPs) are bony growths or deposits, also referred to as bone spurs, osteochondral nodules, osteochondrophytes, or chondro-osteophytes. They are bony projections that form along joint margins, initially as cartilage outgrowths and subsequently endochondral ossification [88] and are a defining feature of OA [6]. OPs usually develop as a reparative response by the remaining cartilage in a damaged joint, correlating with cartilage loss elsewhere in the joint. However, the initial stimuli for OP formation are still not well understood, but humoral [89] and mechanical [90] factors are believed to be involved. Studies also found that growth factors such as transforming growth factor beta (TGFβ), bone morphogenetic protein (BMP) and insulin like growth factors (IGF-1) can potentiate OP development [91-93]. Meanwhile, joint instability has been introducedd as a biomechanical trigger to OP formation [94]. The formation of OPs has been interpreted as an adaptation of the joint to the altered biomechanics of OA joints [95, 96], and to cope with instability and protect articular cartilage by playing a compensatory role in the redistribution of forces to protect articular cartilage [97]. A previous study reported that removing OPs from the arthritic joint seriously increased the varus-valgus motions [98], indicating that OPs may serve some purpose in stabilising an osteoarthritic joint. On the other hand, OPs of the knee predict pain more accurately than the JSN in radiological views [99], suggesting a detrimental effect of OP. Thus, it is still debatable of the role that OPs play in the progression of OA. Overall, the role of OPs in OA progression remains unclear.

***X-ray OPs***

Despite of the widespread use of tomographic imaging tools, conventional radiographs remain the current standard for establishing a radiographic diagnosis of osteoarthritic changes in the knees. JSN and OP formation are two radiographic hallmarks of OA. Radiographic OPs are most prominent medially. On a typical plain x-ray image, they are usually seen anteriorly and medially at the distal femur and proximal tibia, and posteriorly at tibia and patella [100].

Higher OP scores are modestly associated with greater risk of OA progression [101], but the association disappears after further adjustment for limb alignment. OPs are a weaker predictor of OA progression than JSN [102], which is a strong predictor of OA progression in more than 1500 patients. Several studies concluded that there are only modest correlations between knee radiographic OPs and clinical features [47], and radiographic OPs may not be a favorable surrogate for clinical outcome in established OA [103]. Figure 1.2 shows an example of x-ray OPs from our study population.

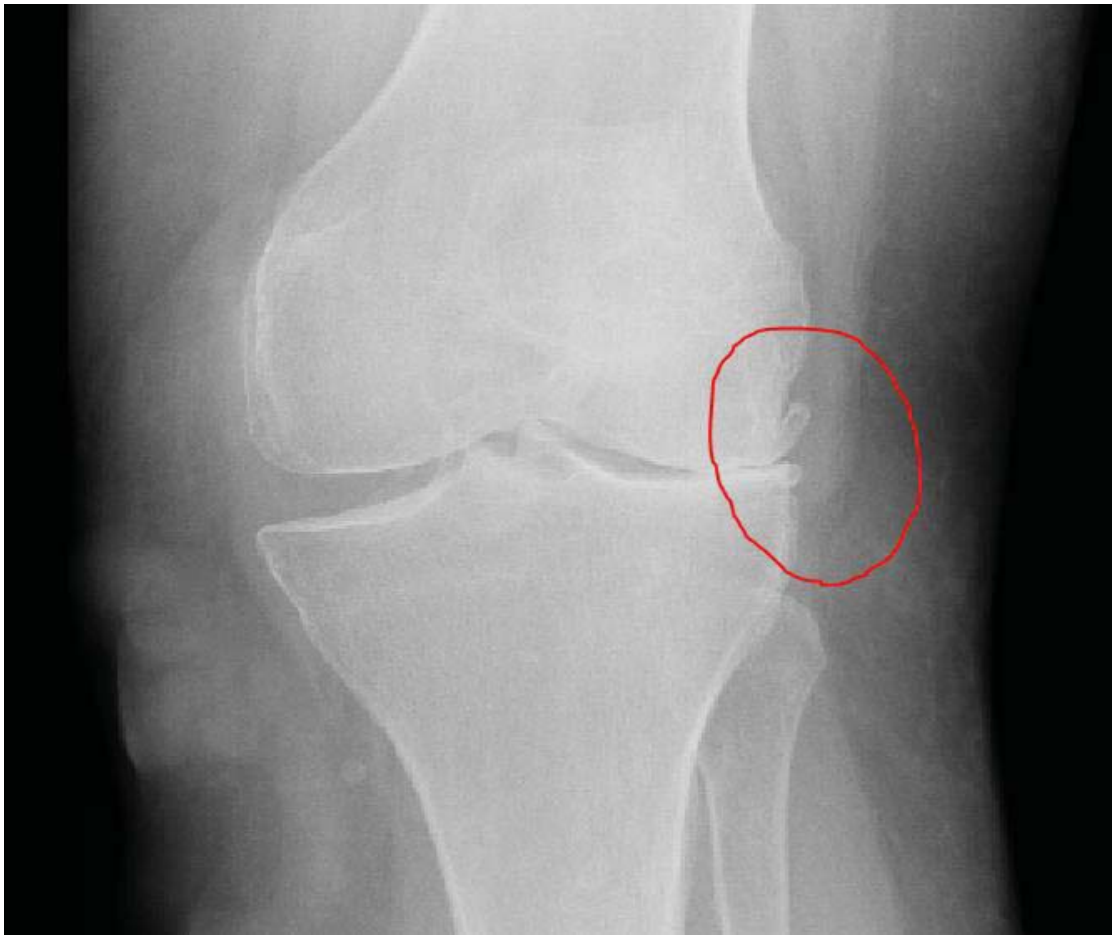


**Figure 1.2** Sample of X-ray OPs

***MRI OPs***

Magnetic resonance imaging (MRI) is a non-invasive multiplanar tomographic tool which provides high-resolution visualization of menisci, synovium, BMLs, articular cartilage, subchondral cysts, intra-articular ligaments and intra-articular lesions that are not detectable by conventional radiographs [104]. MRI assesses OPs in locations that are not easily visualised by conventional radiographs [105], and have higher sensitivity than conventional radiographs for early OP detection [106]. One study revealed that nearly 74% of MRI-detected OPs were present in radiographically normal knees [107]. In contrast, the prevalence of radiographic OPs was approximately 10% in a generally older population (mean age 61 years) [108]. These figures were largely in line with our own dataset which was a population-based older adult cohort study. In our sample, 85%

had MRI-detected OPs at baseline, whereas less than 10% had radiographic OPs [109]. This indicates that MRI is far more sensitive than X-ray to detect OP at early formation and can pick up OPs in locations that not easy to be observed by radiographs. It is important to detect preclinical OA as early interventions could then be introduced to prevent disease progression. Considering that OP formation is one of the criteria for OA, and radiography misses out a large proportion of early OP formations which can be detected by MRI, there must be a large number of early OA patients who have MRI-early OPs but are misclassified as normal on conventional radiographs. To date, the relevance of MRI-early OPs for the development of structural and clinical abnormalities is still uncertain. If MRI-early OPs are proved to be related to future OA progressions, then detections of MRI-early OPs and interventions targeting them are essential and necessary. Figure 1.3 gives an example of an OP that was visible on MR imaging but not on radiography.

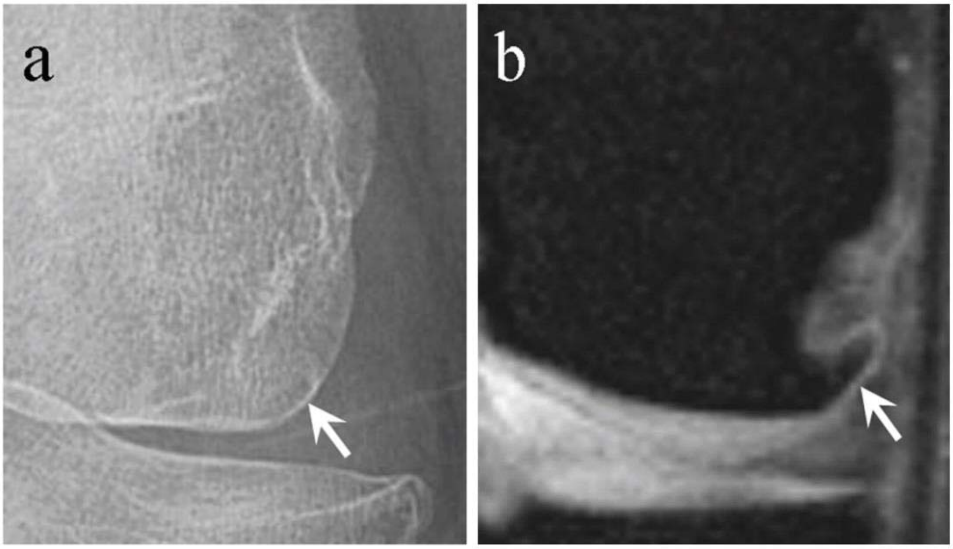



**Figure 1.3** Example of an osteophyte that was not visible on radiographic but was on MRI

*Source: Hayashi et al. "Pre-radiographic osteoarthritic changes are highly prevalent in the medical patella and medial posterior femur in older persons: Framingham OA Study". In: Osteoarthritis and Cartilage, 22.1(2014), 76-83.*

Larger MRI-detected OPs are associated with higher K/L score cross-sectionally [63] and increased knee pain longitudinally [110], and cross-sectional studies suggest that increasing size and presence of MRI-detected OPs were associated with severity of

knee OA [9, 63, 111]. However, there are only two longitudinal studies, which report inconsistent results [110, 112]. The first did not find any significant associations between MRI-detected OPs and knee structural progression [110]. The second was a nested case-control study reporting that subjects with 6 or more locations affected by OPs had 4.4-fold increased odds of being both radiographic and pain progression compared with 0-2 locations affected [112]. The reasons for the discrepancy in the findings are unknown, but more longitudinal studies are needed to elucidate the relationship between MRI-detected OPs and clinical changes of OA.

## 1.3 Cartilage abnormalities

### 1.3.1 Cartilage volume

High-resolution MRI has been used to quantify cartilage volume in OA [113, 114]. The most common technique involves estimation of the volumes of individual cartilage plates by manually drawing cartilage contours around the cartilage boundaries section by section, which are then reassampled by means of bilinear and cubic interpolation to give a total volume. Examples of segmentation are illustrated in Figure 1.4

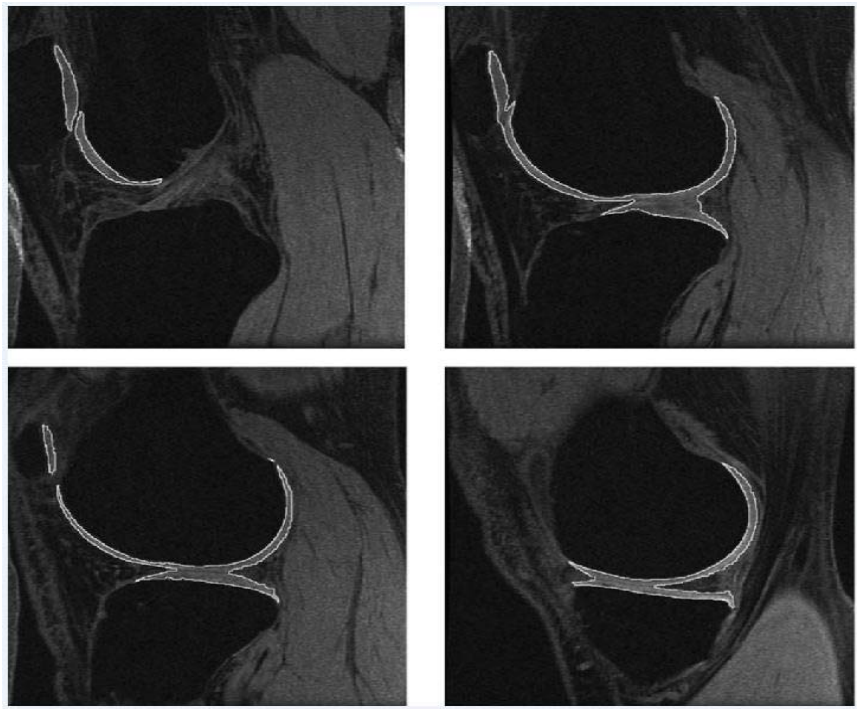


**Figure 1.4** Sample of cartilage volume segmentation

*Source: Grau et al. "Improved watershed transform for medical image segmentation using prior information". In: IEEE Trans Med Imaging 23.4 (2004), pp. 447-458*

The relationship between cartilage volume and radiographic grade of OA has been reported broadly but longitudinal associations are uncertain. Cicuttini et al. [115] found that the inverse association between JSN score and cartilage volume was stronger than that between OPs score and cartilage volume. Jones et al. [116] reported similar findings in the lateral tibia and patella compartments. In the patellofemoral (PF) joint, significant inverse associations were also found on lateral and skyline PF radiographs [117]. However, only few longitudinal studies directly compared cartilage volume change to quantitative measurements of radiographs, which generated controversial findings. Bruyere et al. [118] reported a significant association between changes in JSN and changes in cartilage volume in knee joint. In contrast, Gandy et al. [119] described no statistical correlation between cartilage volume loss and radiographic changes in any of the knee compartments. In general, a systematic review concluded that there is moderate association between cartilage volume loss and reduced radiographic joint space [120].

The link between cartilage volume and clinical symptoms is also widely reported and associations are consistent. Hunter et al. [45] found a significant negative association of patellar cartilage volume with the total WOMAC knee pain in a cross-sectional study of community-based population. Wluka et al. [121] observed a moderate correlation between tibial cartilage volume and symptoms in a cross-sectional study of early symptomatic knee OA. On the other hand, Raynauld et al. [119] reported no association between cartilage volume loss and worsening of symptoms. One study revealed that for each 1% increase rate of tibial cartilage volume loss, there was a 20% increase risk of knee replacement four years later [122]. Overall, the measurement of cartilage volume using MRI can provide longitudinal quantification of cartilage loss and assess clinical progressions of OA [123].

### 1.3.2 Cartilage defects

MRI can also visualize knee cartilage defects directly. There are several grading systems to assess the severity of cartilage defects using MRI, such as Whole Organ MR Scoring (WORMS) systems and Boston-Leeds Osteoarthritis Knee Scoring (BLOKS) [124, 125]. Our previous studies graded cartilage defects using a validated classification system that based on following criteria. Grade 0, normal cartilage; grade 1, focal blistering and intracartilaginous low signal intensity area with an intact surface and bottom; grade 2, irregularities on the surface or bottom and loss of thickness of less than 50%; grade 3, deep ulceration with loss of thickness of more than 50%; grade 4, full-thickness cartilage wear with exposure of subchondral bone [126-128]. A cartilage defect had to be present in at least two consecutive slices. This grading system is also used in current thesis. Figure 1.5 illustrated examples of cartilage defect grading.

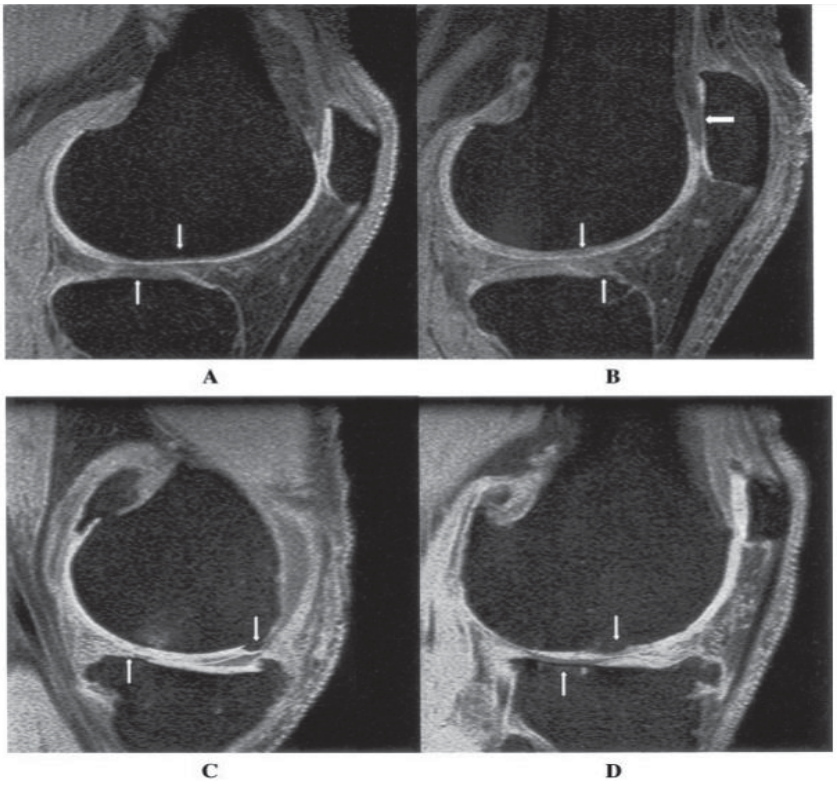



**Figure 1.5** Representative sagittal T1-weighted fat-suppressed 3-dimensional magnetic resonance images illustrating cartilage defects grading

**A**, normal patellar cartilage but with cartilage defect of grade 1 at tibial (up arrow) and femoral (down arrow) sites. **B**, Cartilage defects of grade 2 at tibial site (up arrow), of grade 1 at femoral site (down arrow), and of grade 3 at patellar site (left arrow). **C**, cartilage defects of grade 3 at tibial (up arrow) and femoral (down arrow) sites. **D**, cartilage defects of grade 4 at tibial site (up arrow) and of grade 2 at femoral site (down arrow).

*Source: Cicuttini et al. "Association of cartilage defects with loss of knee cartilage in healthy, middle-age adults: a prospective study". In: Arthritis & Rheumatology, 52.7 (2005), 2033-9.*

Cartilage defects are common and highly variable. In a cohort study of predominantly non-osteoarthritic adults, about 20% of participants had increased cartilage defects in total knee compartment over 2 years [129], while in older healthy participants, about two-thirds had increased cartilage defects over 2 years [128]. In patients with symptomatic OA, 46% had increased cartilage defects in the medial tibiofemoral compartment and 22% had increased cartilage defects in the lateral tibiofemoral [130]. Interestingly, other studies report reduction in cartilage defect grade over time [128, 129, 131]. It is unclear if this is physiological or whether related to other factors such as measurement error. Two high-quality cohort studies of healthy, community-based participants reported an inverse relationship between physical activity and cartilage

defects, indicating beneficial effects of physical activity [132, 133]. According to a study that investigated the natural history of cartilage defects among knee OA patients, the factors related to cartilage defect change over 2 years included age, sex, BMI, baseline cartilage defect score and baseline tibial plateau bone area. Height, weight were not related to cartilage defect change [134].

Many studies have examined the predictive value of cartilage defects to MRI-detected OA progression. One reported that baseline cartilage defect scores at all knee compartments had a dose-response relationship with change of cartilage volume at the corresponding site [127]. Additionally, decreased knee cartilage defect score was associated with increased cartilage volume at all compartments [135]. Another study revealed that increased cartilage defect score was significantly related to baseline tibiofemoral OP, tibial bone area, and medial tibiofemoral and patellar cartilage volume [129]. Prevalent knee cartilage defects were also correlated to BMLs [136, 137], meniscal tears [136] anterior cruciate ligament tears [131], and synovial thickening [138]. These studies suggested that cartilage defect is a solid predictor of MRI-detected structural progression of OA overtime.

There is consistent evidence showing that cartilage defects are significantly associated with pain, and this is largely independent of other structural features [23, 67, 84, 139]. In a population-based cohort study, medial tibial cartilage defects scores were independently associated with severity of knee pain [23]. Moreover, a dose-response relationship was also found between knee pain severity and the number of sites having grade 3 or 4 cartilage defects [23]. The majority of associations were independent of radiographic OA and BMLs [23, 111]. Knee cartilage defects also predict total knee replacement (TKR). In a cohort of 117 subjects with symptomatic knee OA, higher total cartilage defect scores conferred a 6-fold increased risk of TKR over 4 years compared with those lower scores [140]. Data from the Osteoarthritis Initiative (OAI) showed that baseline cartilage defect scores were the strongest independent predictors of subsequent knee replacement [141]. In general, these studies suggested that cartilage defect can serve as a promising biomarker to identify OA patients at risk for disease progressions.

## 1.4 Systematic inflammation

### 1.4.1 Interleukins

Despite OA being traditionally regarded as a non-inflammatory arthritis, growing evidence has shown that the clinical course of OA may be largely driven by systemic and localized inflammation [26, 142], albeit at much lower levels than the recognized inflammatory arthropathies [10, 143]. Cytokines such as interleukin 1(IL-1), IL-6, IL-8 and TNF-α are related to morphological changes including cartilage erosion and synovial inflammation [144-146]. Moreover, a relatively insufficient production of natural antagonist of IL-1 receptor in OA synovium could be associated with over production of nitric oxide in OA tissues [147]. In the pathophysiology of OA, interleukins play important roles in synovitis, cartilage destruction and pain [148, 149]. IL-6 is a 184 amino acid residue protein [149] which has both pro- and anti-inflammatory effects both inside and outside of the joint [150, 151]. Although emerging evidence suggests that low-level systemic inflammation is involved in OA pathogenesis [26, 152], so far the role of IL-6 in OA process remains debatable. In a spontaneous ageing model of OA, mice deficient in IL-6 have higher levels of cartilage loss, suggesting a potentially protective role of IL-6 in the development of OA [153]. By contrast, higher serum IL-6 concentrations were related to higher prevalence of OPs in older adults [142] and circulating levels of IL-6 have been related to prevalence of knee OA [143].

On the other hand, some interleukins have anti-inflammatory effects. IL-4, IL-10 and IL-13 are among those interleukins that can modulate various inflammatory processes [154, 155]. Their anti-inflammatory properties appear to largely depend on the target inflammatory cytokines. For example, IL-4 can surpress synthesis of both TNF- α and IL-1β in the same manner as low-dose dexamethasone [156]. Similarly, IL-10 can inhibit the synthesis of TNF-α and IL-1 [157], and IL-13 significantly inhibits lipopolysaccharide (LPS)-induced TNF- α production by mononuclear cells from peripheral blood, but not in cells from inflamed synovial fluid [158]. Although the applicability has yet to be proven, these anti-inflammatory interleukins can have potential for OA treatments.

T-helper 17 (Th-17) cells have been identified as a subset of CD4 (+) T helper (Th) cells that is differentiated from Th1 and Th2 cells [159, 160], and involved in a number of inflammatory and autoimmune diseases such as multiple sclerosis, rheumatoid arthritis (RA), psoriasis and inflammatory bowel disease [161, 162]. IL-17A and IL-17F are the prototypical members of the IL-17 cytokine family, which are produced by Th-17 [163]. They share the most homology at the amino acid level and have overlapping but also distinct effector functions on autoimmune diseases [164, 165]. Cytokines such as IL-1, IL-6, IL-23 and TGF-β can promote the differentiation of CD4 (+) cells, leading to the expression of IL-17A, IL17F and IL-6 [166, 167].

Apart from playing critical roles in the aetiology and progression of autoimmune diseases, these Th-17 family interleukins also play crucial roles in the initiation of local inflammatory reaction [168] and cartilage destruction [169]. Previous studies revealed that IL-17A induces production of IL-1β, IL-6 and TNF-α, the major pro-inflammatory cytokines in OA [170]. Chen et al. [171] reported a positive association between knee OA severity and IL-17 concentration in synovial fluid, but not in serum. Liu et at [172] demonstrated that synovial IL-17 level was correlated with severity of knee pain, but not with ROA severity. These studies have limitations such as cross-sectional design, small sample size and lack of adjustment for potential confounders. A recent case-control study reported that increased serum level of IL-17A and IL-23 were related to increased pain in OA patients [173]. To date, however, longitudinal studies looking at the roles of Th-17 family interleukins on OA aetiology are still rare.

### 1.4.2 hs-CRP

C-reactive protein (CRP) is a circulating marker of systemic inflammation. It is synthesized by hepatocytes and adipocytes and can be regulated by pro-inflammatory cytokines [174]. In 1992, an immunoassay was introduced by Montagne et al. to perform high sensitive CRP (hs-CRP) measurement [175], which reflected low grade systemic inflammation and was not able to be detected using previous method. Only 1 year later, a study reported that CRP concentrations are elevated in OA patients but to a lesser extent than in RA patients [176]. Since then, numerous studies have explored the relationship between systemic or local level of CRP and OA onset using multiple

methodologies. Studies have found that comparing with healthy controls, circulating levels of hs-CRP are significantly higher in OA patients [177-179]. A meta-analysis concluded that there was a moderate but statistically significant association between hs-CRP levels and increasing pain score, and worsening physical function; however, this meta-analysis concluded hs-CRP level was not associated with K/L scores or JSN [180].

With regards to the predictive value of hs-CRP for OA progression, the findings from studies are controversial. The study by Sharif et al. [181] revealed higher serum level of CRP in participants with progression of knee OA compared with controls. This was not supported by Rotterdam Study with a much bigger dataset, which showed lower serum levels of CRP in participants with progression of knee OA, compared with controls [182]. Sower et al [183] evaluated CRP concentration as a potential biomarker of prevalence and incidence of knee OA, and found that women with knee OA onset over 2.5-year had significantly higher CRP concentrations; however, its high dependence on obesity limits its utility as an exclusive biomarker for knee OA. Kerkhof et al. [184] conducted another meta-analysis and concluded no evidence of links between serum CRP concentrations and OA independent of BMI.

Studies regarding associations between CRP and BMLs are rare. One cross-sectional study with a limited sample size (n=30) found no significant differences in hs-CRP levels between patients with and without BMLs, but this may be due to type 2 errors from that small sample size [185]. Another cross-sectional study with 194 participants involved showed that in patients with the highest quartile of hs-CRP, IL-17 was positively associated with BMLs at femoral compartment [186]. Therefore, longitudinal studies are required to determine the associations between CRP and BMLs in the development and progression of OA.

## 1.5 Summary

OA is one of the leading cause of pain, loss of function and disability amongst the elderly. Knees are the most commonly affected joints [2]. OA constitutes 63% of hospital inpatient expenditure while RA just 3.5%. Moreover, OA account for 30% of

hospital outpatient expenditure, in contrast of 16.3% attributed to RA. It is predicted that 7 million Australians will suffer from arthritis by 2050, among which OA contributes the largest proportion [187].

Emerging evidence suggests that subchondral bone changes play vital roles in the onset and progression of OA. BMLs and OPs are typical subchondral bone changes that are pathological and related to OA [109]. There are very few clinical studies examining BMLs at the specific site of PFJ, and the natural history of PFJ BMLs has not yet been described. Additionally, whether systemic inflammation involved in the aetiology of BMLs in OA patients is unknown. OPs visible on radiographs have been viewed as a hallmark and defining structural feature of OA since 1950s; however, the predictive value of radiographic OPs on OA symptomatic and structural progression is poor, with only modest correlations between knee OPs and clinical features of OA [47, 103]. On the other hand, MRI-detected OPs can assess OPs in locations that are not easily visualised by radiographs [105, 188], and have higher sensitivity than conventional radiographs for detection of early OP formation [104].

The following chapters describe the natural history of BMLs at the rarely studied PFJ compartment and evaluate the role of systemic inflammations in the aetiology of BMLs. Furthermore, cross-sectional and longitudinal relationships between MRI-detected OPs and knee structural abnormalities as well as knee pain change are investigated in the following chapters.

# Chapter 2: Research Questions

The following research questions are summarised based on background and rationales introduced in Chapter 1 of this thesis.

1. To describe the natural history of patellofemoral joint (PFJ) BMLs over 2.6 years and longitudinal associations between change in PFJ BMLs over 2.6 years, knee pain change over 5 years and baseline knee cartilage morphology in older adults.

a) Is BML at PFJ static or variable over 2.6 years?
b) Do changes in PFJ BMLs over 2.6 years correlate with knee pain changes over 5 years?
c) Can baseline cartilage morphology predict PFJ BMLs changes over 2.6 years?

2. To evaluate the cross-sectional and longitudinal associations between serum level of high sensitivity C reactive protein (hs-CRP) at baseline, knee BMLs and knee pain in patients with knee OA at baseline and changes over 2 years.

a) What are the cross-sectional and longitudinal associations between baseline serum hs-CRP and baseline as well as change of knee BMLs over 2 years in patients with symptomatic knee OA?
b) What are the cross-sectional and longitudinal associations between baseline serum hs-CRP and baseline as well as change of knee pain in patients with symptomatic knee OA?

3. To examine the cross-sectional and longitudinal associations between serum levels of interleukin (IL)-6, IL-17A, IL-17F, IL-23 and knee BMLs in patients with knee OA.

a) What are the cross-sectional and longitudinal associations between baseline serum autoimmunity-related interleukins and baseline as well as change of knee BMLs in patients with symptomatic knee OA?

4. To investigate the cross-sectional and longitudinal associations between baseline MRI-detected OPs and knee structural abnormalities over 2.6 years as well as knee pain over 5 years in older adults.

a) What is the prevalence and distribution of MRI-detected OPs in generally older population?

b) Can baseline MRI-detected OPs predict structural and symptomatic progression of OA over 2.6 years?

5. To describe the prevalence of MRI-OPs that can be detected by MRI but not by x-ray, and to evaluate the longitudinal associations with knee pain change over 5 years and structural changes over 2.6 years.

a) Is MRI-OPs common in a generally older population?

b) Can MRI-OPs serve as a biomarker that predicts progression of OA over time?

# Chapter 3: Methodology

## 3.1 Prelude

This chapter delineates the study population and design for the Tasmania Older Adult Cohort (TASOAC) and Vitamin D Effect on Osteoarthritis (VIDEO) studies as well as protocols of exposure and outcome measurements. These measurements are common in the subsequent chapters. Chapter 4, 7 and 8 stemmed from analyses using the data from TASOAC study. Chapter 5 and 6 arose from analyses using the data from VIDEO study. Data chapters are presented in the form in which they were submitted to, or accepted by peer-reviewed journals for publication. Due to requests from journal reviewers, there were some differences in the presentation of methods, analyses, results, and interpretations throughout these chapters.

## 3.2 Tasmanian Older Adult Cohort (TASOAC) Study design

### 3.2.1 Study design

The TASOAC study is an ongoing, prospective, population-based cohort study that aims at identifying the genetic, environmental, and biochemical factors associated with the development and progression of OA. Baseline data collection were ascertained from April 2002 to September 2004. The follow-up study was carried out at 2.7 years later (range 2.6-3.3 years), with pain assessment performed also at a second follow-up at about 5 years later (range 5.3-6.8 years).

### 3.2.2 Ethics

The Southern Tasmanian Health and Medical Human Research Ethics Committee approved the study (Ethics Approval Number: H6488). Written informed consent was obtained from all participants.

### 3.2.3 Study population

TASOAC consisted of older adults between the ages of 50 and 80 (mean: 62 years; standard deviation (SD): 7 years). Participants were randomly selected from the electoral roll of Southern Tasmania. The electoral roll represents the most complete population listing of Australian adults available, as voting is compulsory in state and federal elections. Sex stratified simple random sampling was used to provide equal number of males and females in the final cohort. Equal distribution was also drawn from rural and urban regions in Southern Tasmania. Institutionalized older adults were excluded as TASOAC study was designed to investigate community dwelling older adults. Participants with contraindications for MRI (including iron filings in the eye, claustrophobia, metal suture and presence of shrapnel) were also excluded. 2135 eligible participants were identified initially, from which 1100 participants were enrolled in the study. The MRI scanner was decommissioned halfway through the phase 2 follow-up period, resulting in only about half of the phase 2 participants with follow-up MRI images.

### 3.2.4 Demographic characteristics

Age, gender, race, education and occupation were recorded and collected by questionnaires at baseline.

### 3.2.5 Anthropometrics

Weight was measured with shoes, socks and bulky clothing removed using electronic scales (nearest 0.1kg). Height was measured with shoes, socks and headgear removed using a stadiometer (nearest 0.1cm). Body mass index (BMI) was calculated using height and weight ($kg/m^2$).

### 3.2.6 Radiographic measurements

A standing anteroposterior semiflexed view of the right and left knee was performed with 15°of fixed knee flexion was performed in all subjects at baseline and scored individually for osteophytes (OPs) and joint space narrowing (JSN) on a scale of 0-3 (0=normal and 3=severe) based on the Altman atlas as previously described [189]. Medial tibiofemoral or lateral tibiofemoral JSN was scored separately. OPs were scored

at each site of medial tibia, medial femur, lateral tibia and lateral femur. Each score was determined by consensus of two readers who simultaneously assessed the radiograph with immediate reference to the atlas. The prevalence of medial or lateral tibiofemoral JSN or OPs was defined as the presence of radiographic osteoarthritis (ROA) [190].

### 3.2.7 MRI measurements

***Magnetic Resonance Imaging***

MRI scans of the right knees were performed on two phases (baseline and 2.6 years later) and scanned in the sagittal plane on a 1.5-T whole body magnetic resonance unit (Picker, Cleveland, OH, USA) using a commercial transmit-receive extremity coil. The image sequences were used as follow: (1) T1-weighted fat saturation 3D gradient recall acquisition in the steady state; flip angle 30°; repetition time 31 ms; echo time 6.71 ms; field of view 16 cm; 60 partitions; 512×512 matrix; acquisition time 11 min 56 s; one acquisition. Sagittal images were obtained at a partition thickness of 1.5 mm and an in-plane resolution of 0.31×0.31 (512×512 pixels). (2) a T2-weighted fat saturation 3-D fast spin echo, flip angle 90, repetition time 3067 ms, echo time 112 ms, field of view 16 cm, 15 partitions, 228x256-pixel matrix; sagittal images were obtained at a partition thickness of 4 mm with a between-slices gap of 0.5 to 1.0 mm. The image database was transferred to a computer workstation using the software program Osirix (University of Geneva, Geneva, Switzerland) as previously described [116, 191].

***Cartilage defects***

Knee cartilage defects were measured by a trained observer on T1-weighted MR image at baseline and 2.6-year follow-up according to previously described criteria [126, 192]. Grade 0, normal cartilage; grade 1, focal blistering and low-signal intensity change with an intact surface and bottom; grade 2, irregularities on the surface or bottom and loss of thickness of less than 50%; grade 3, deep ulceration with loss of thickness of more than 50%; grade 4, full thickness cartilage loss with exposure of subchondral bone [126]. The worst score of each individual site in the relevant compartment (or whole knee) was regarded as the cartilage defect score in that compartment (or whole knee). The reader was blinded of the initial results at the time of the second reading. The presence of cartilage defects was defined as a cartilage defect score of ≥2 at any site.

An increase in cartilage defects was defined as a change of cartilage defect score ≥1. Intra-observer reliability (expressed as ICC) was 0.89-0.94 and inter-observer reliability was 0.85-0.93 [126].

***Cartilage volume***

Knee cartilage volume was measured on T1-weighted images by a single trained observer [126]. The volumes of individual cartilage plates (medial tibial, lateral tibial, medial femoral, lateral femoral, patellar and trochlear) were isolated from the total volume by manually drawing disarticulation contours around the cartilage boundaries on a section by section basis. These data were then reassembled by means of bilinear and cubic interpolation (area of 312×312 um and 1.5 mm thickness, continuous sections) for the final 3-dimentional rendering. The volume of patellofemoral and tibiofemoral compartments were obtained by summing the pertinent cartilage plates within compartments. Changes in cartilage volume were calculated as: percentage change per annum= [(follow-up volume-baseline volume)/baseline cartilage volume]/time between 2 scans in years×100. The coefficient of variation (CV) for cartilage volume assessments was 2.1% to 2.6% [193, 194].

***Subchondral BMLs evaluation***

Subchondral BMLs were defined as discrete areas of increased signal adjoining to the subchondral bone on T2-weighted MR images and were scored at medial tibial, lateral tibial, medial femoral, lateral femoral, medial patellar and lateral patellar regions using Whole-Organ Magnetic Resonance Imaging Score (WORMS) [125]. Grade 0, absence of BML; grade 1, area smaller than 25% of the region; grade 2, area between 25% to 50% of the region; grade 3, are larger than 50% of the region. Each BML was scored according to the maximal percentage of bone are that the lesion occupied on the slice. The worst score of each individual site in the relevant compartment (or whole knee) was regarded as the BML score in that compartment (or whole knee). PFJ compartment BMLs were calculated by summing BML scores of patella and trochlea (anterior femoral). An increase in BMLs was deemed as a change in BMLs of ≥1 from baseline to follow-up at certain compartment. The inter-reader reliability of current BML scoring system was excellent [71, 195].

***Osteophytes***

MRI-detected osteophytes (OPs) were measured according to the Knee Osteoarthritis Scoring System [196] where OPs are defined as focal bony excrescences, seen on sagittal, axial or coronal images, extending from a cortical surface. OPs were assessed using the following scale: grade 0, absent; grade 1, minimal (<3 mm); grade 2, moderate (3-5 mm); grade 3, severe (> 5 mm). Size was measured from the base (distinguished from that of adjacent articular cartilage with a normal MRI appearance) to the tip of OP [188] at each of the following 14 sites: the anterior (a), central weight-bearing (c) and posterior (p) margins of the femoral condyles and tibial plateaus, and the medial (M) and lateral (L) margins of the patella [125]. The worst score of each individual site in the relevant compartment (or whole knee) was regarded as the OP score in that compartment (or whole knee). MRI-detected OP was considered as present if OP score of ≥ 1. Intra-rater reliability (expressed as ICC) was 0.94-0.97 and inter-rater reliability was 0.90-0.96.

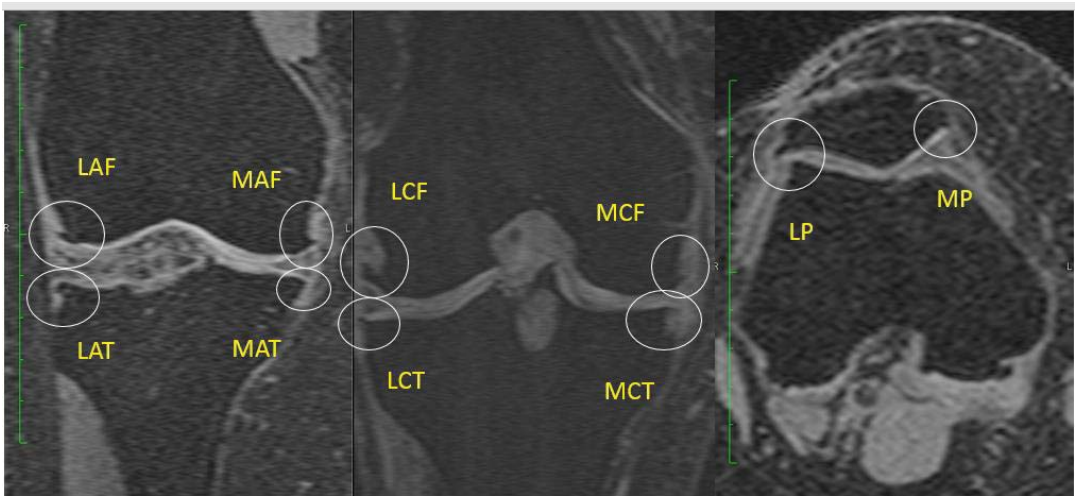



**Figure 3. 1** Examples of osteophytes assessed at different sites on MRI

LAF=lateral anterior femoral (grade 1); LAT=lateral anterior tibial (grade 2); MAF=medial anterior femoral (grade 1); MAT=medial anterior tibial (grade 0); LCF=lateral central femoral (grade 3); LCT=lateral central tibial (grade 1); MCF=medial central femoral (grade 1); MCT=medial central tibial (grade 1); LP=lateral patella (grade 3); MP=medial patella (grade 0).

***Meniscal damage***

Meniscal damage was measured on T1-weighted MR images as previously described [197]. For meniscal tears, the following scale was applied: 0 = no damage; 1 = one of three meniscal areas involved (anterior, middle, and posterior horns); 2 = two of three areas involved; 3 = all three areas involved. For meniscal extrusions, the extent of meniscal extrusion on the medial or lateral edges of the tibiofemoral joint space, not including the osteophytes, was evaluated for the anterior, middle, and posterior horns of the mensci in which 0 = no extrusion, 1 = partial meniscal extrusion, and 2 = complete meniscal extrusion with no contact with the joint space. Anterior, posterior and middle scores were summed to get medial and lateral meniscal tear/extrusion scores. The worst meniscal tear/extrusion score of medial or lateral compartment was regarded as total meniscal tear/extrusion score. The intra- and inter- rater correlation coefficient ranged from 0.86 to 0.96 for meniscal tear and 0.85 to 0.92 for meniscal extrusion [198].

***Bone size***

Knee tibial plateau bone areas were assessed from T1 MRI using an independent work station and the software program Osiris unpaired and unblinded to sequence [116]. Medial and lateral tibial plateau bone areas were uniform in nature and thus can be measured directly from the reformatted axial images. The CVs for these measures are 2.2-2.6% [189].

### 3.2.8 WOMAC pain assessment

Knee pain was measured at knee level using Western Ontario McMaster Osteoarthritis Index (WOMAC) [23] using a 10-point scale from 0 (no pain) to 9 (most severe pain) at baseline, 2.6 and 5 years later. The 5 subscales (walking on flat surface, going up/down stairs, at night, sitting/lying and standing upright) were assessed separately and summed to create a total pain score (0 to 45). Change in knee pain score was calculated as follow-up value – baseline value. An increase in total WOMAC pain was defined as a change in WOMAC pain score of $\geq 1$.

## 3.3 Vitamin D Effect on Osteoarthritis (VIDEO) Study design

### 3.3.1 Study design

VIDEO is a multicentre, parallel group randomised, placebo-controlled and double-blinded clinical trial which aims to examine the effects of vitamin D supplementation on knee pain and structural changes in patients with symptomatic knee OA and low 25-hydroxyvitamin D (25OHD) level. The inclusion and exclusion criteria are listed as follows:

***Inclusion criteria:***

a) Age 50-79 years old;
b) Meet the American College of Rheumatology (ACR) criteria [6] of symptomatic knee OA diagnosis by an experienced rheumatologist;
c) Men and women with symptomatic knee OA for at least 6 months with a pain visual analogue scale (VAS) of at least 20 mm;
d) Relatively good health (0-2 according to the investigators global assessment of disease status on a 5-point Likert scale, range 0[very well] to 4 [very poor]);
e) An ACR functional class rating of I, II and III [199];
f) Serum vitamin D level of >12.5 nmol/L and <60 nmol/L

***Exclusion criteria:***

a) Severe knee ROA (grade 3 according to Altman's atlas [34]);
b) Severe knee pain (more than 80 mm on a 100-mm VAS);
c) With hypersensitivity to vitamin D;
d) With rheumatoid arthritis, psoriatic arthritis, lupus, or cancer;
e) With severe cardiac or renal function impairment;
f) With any condition possibly affecting oral drug absorption (gastrectomy or clinically significant diabetic gastro-enteropathy);
g) Significant trauma to the knees including arthroscopy or significant injury to ligaments or menisci of the knee within 1 year preceding the study;
h) Having taken vitamin D supplementation in last 30 days;
i) Need for knee or hip surgery in the next 2 years;

### 3.3.2 Study population

The VIDEO study was conducted in Melbourne and Hobart, Australia. Sample size calculation had been done before patient recruitment and 200 patients in each arm (allowing fo a 20% dropout over 2 years) were calculated to be sufficient to detect the differences between treatment groups [52, 200]. Sample size calculation assumed $\alpha$=0.05 and $\beta$=0.20, and was performed using the Cohen formula [201]. Participants were recruited using various strategy, including collaboration with general practitioners, specialist rheumatologists, and orthopaedic surgeons. A total of 599 participants were screened for from 5 Jun 2010 to 1 Dec 2011, with 413 participants recruited for randomization. Figure 3.2 illustrates outlines of participant recruitment and withdrawal during study period.

192 participants (mean 63 years, range 49-79 years, female 53%) in Hobart were selected from VIDEO study population to have hs-CRP and other inflammatory interleukins measurements.

### 3.3.3 Ethics

The Tasmania Health and Human Medical Research Ethics Committee (reference number H1040) and Monash University Human Research Ethics Committee (reference number CF10/1182-2010000616) approved this study. Informed written consent was obtained from all participants.

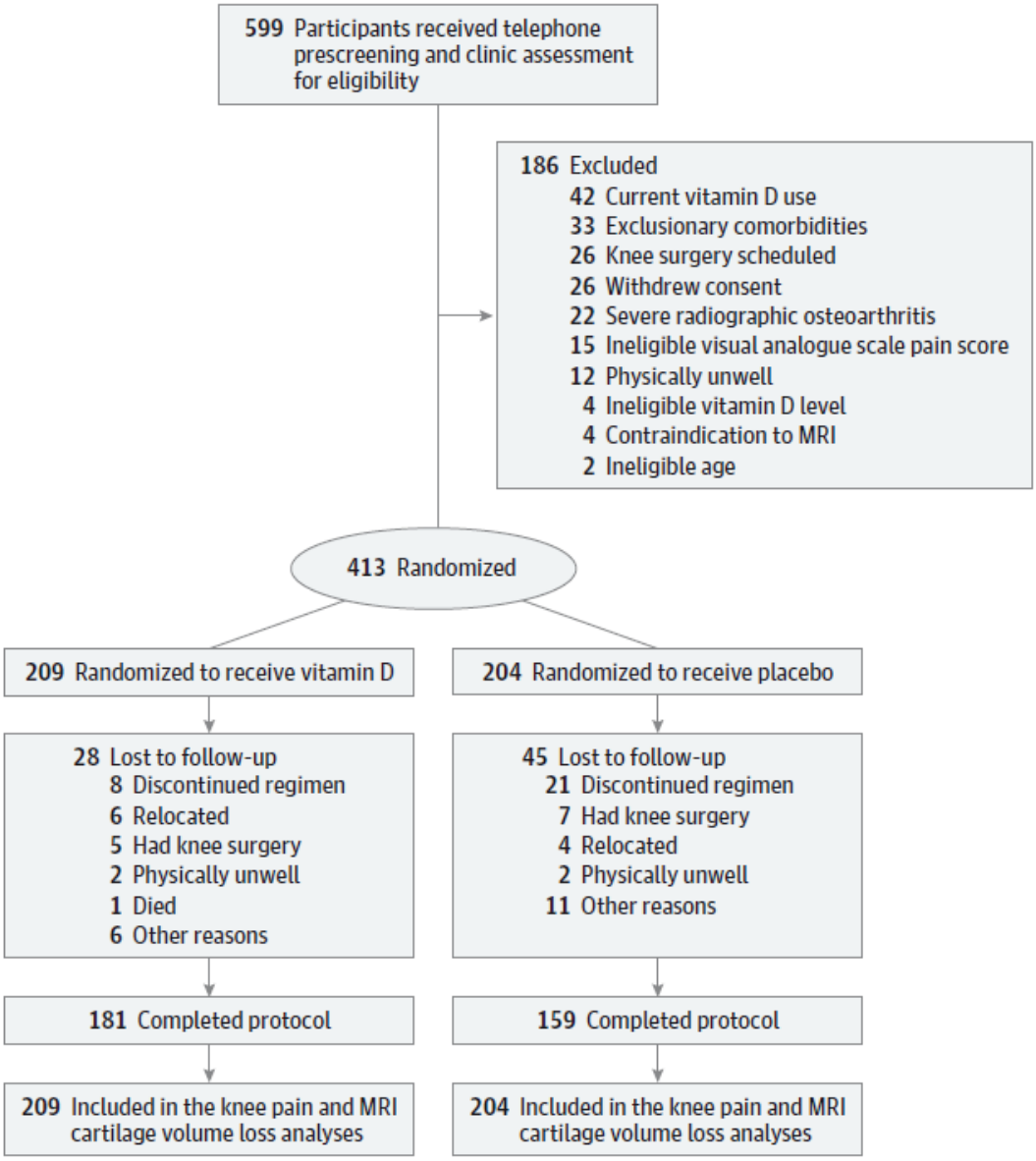



**Figure 3. 2** Flow chat of participant recruitment in VIDEO study

*Source: Jin et al "Effect of vitamin D supplementation on tibial cartilage volume and knee pain among patients with symptomatic knee osteoarthritis" in JAMA 315.10 (2016), 1005-13*

### 3.3.4 25OHD assays

In the original RCT study, after randomisation, participants in the intervention and control groups were given a monthly capsule of 50,000 IU (1.25 mg) vitamin D3 (cholecalciferol) and placebo, respectively, for 24 months in total [202]. Serum 25OHD was assayed by Liaison method utilizing a direct competitive chemiluminescent

immunoassays (DiaSorin Inc, Stillwater, Minnesota, USA) at screening, month 3 and month 24, The intra-assay and inter-assay coefficients of variation were 3.2% and 6.0%.

### 3.3.5 Serum inflammatory markers measurements

***Hs-CRP***

Serum levels of hs-CRP were assayed at baseline and after 24 months using enzyme-linked immunosorbent assay [203]. The minimal detectable concentration is around 0.02 ug/ml. The coefficients of variation (CVs) in our hands was 5.8%-6.3%. Change in serum levels of hs-CRP was calculated as: follow-up value – baseline value.

***Interleukins***

Serum levels of IL-6, IL-17A, IL-17F and IL-23 were assayed at baseline and after 24 months using enzymelinked immunosorbent assay [10]. The limits of detection are 0.49 pg/ml, 1.84 pg/ml, 14.6 pg/ml and 34.4 pg/ml respectively. The coefficients of variation (CVs) were 5.8%-6.3%.

### 3.3.6 Anthropometrics

Height and weight were measured as described in section 3.2.4, and body mass index (BMI) was calculated accordingly (weight/height$^2$).

### 3.3.7 Demographic characteristics

Age and gender were recorded.

### 3.3.8 WOMAC knee pain assessment

Knee pain was assessed using the WOMAC questionnaire [204] at baseline and 24 months later. Five items were used to assess pain: walking on flat surface, when standing upright, when going up/down stairs, at night in the bed and when sitting/lying. In contrast with TASOAC study, these items were assessed using 100 mm visual analogue scales in VIDEO study. Total pain score (range 0-500) was obtained by

summing up all items. Change in total knee pain was calculated as (follow-up value-baseline value of total pain scores).

### 3.3.9 BMLs assessment

Subchondral BMLs were defined as discrete areas of increased signal adjoining the subchondral bone on T2-weighted MR images and scored at medial tibial, lateral tibial, medial femoral, lateral femoral, medial patellar and lateral patellar regions using a modified version of Whole-Organ Magnetic Resonance Imaging Score (WORMS) [125]. Grade 0, absence of BML; grade 1, area smaller than 25% of the region; grade 2, area between 25% to 50% of the region; grade 3, are larger than 50% of the region. In the modified WORMS, the medial and lateral tibial and femoral compartments are divided into three sub-regions (anterior, central, posterior), and the tibia has an additional spinous sub-regions which represents the portion of the tibia beneath the tibial spines. Each BML was scored according to the maximal percentage of bone area that the lesion occupied on the same slice. The worst score of each individual site in the relevant compartment (or whole knee) was regarded as the BML score in that compartment (or whole knee). An increase in BMLs was defined as a change in BMLs of ≥1 from baseline to follow-up at certain compartment. The intra-rater reliability (ICC=0.93-0.98) and inter-rater reliability (ICC=0.91-0.97) were excellent.

## 3.4 Statistical Analysis

T-tests or $\chi^2$ tests were used to compare means or proportions as appropriate. Standard diagnostic checks of residuals and model fit comparisons were performed routinely. A p value less than 0.05 (two-tailed) was considered statistically significant. All statistical analyses were performed on Stata12.0 for Windows (Stata Corporation, TX, USA) Detailed statistical analyses descriptions are presented in each chapter.

# Chapter 4:

# Patellofemoral bone marrow lesions: natural history and associations with pain and structure

This manuscript has been published (Zhu *et al* Arthritis Care & Research. 2016; 68(11)). The typeset version of the manuscript as it appeared in the journal is in Appendix 1. The text of this chapter is the same as the published version, except where changes have been requested by the examiners. Thus, there is some repetition of the methods.

## 4.1 Introduction

Structural abnormalities in the subchondral bone are key players in the pathogenesis of OA [54]. BMLs are ill-defined hyperintense features seen on magnetic resonance (MR) images. In the whole knee, they precede cartilage pathology, including cartilage defects and cartilage volume loss [85, 126]. BMLs are associated with knee pain [75]and predict disease progression [78, 79]. Incident BMLs and increases in BML size are linked to the development of knee pain in pain-free subjects [76]; and increases in BML size are associated with increasing knee pain over time [77]. Kothari et al. [61]and Aitken et al. [205] reported that baseline BMLs were associated with cartilage loss in the same subregion. Raynauld et at. [71] Found that an increase in BML size was associated with cartilage volume loss in the medial but not in lateral compartment.

The natural history of BMLs has been reported in some longitudinal studies. The MOST (Multi-centre Osteoarthritis) study [72]showed the one-third of knees without baseline BMLs developed new lesions at follow-up, 50% of the prevalent BMLs either regressed or resolved. Previous analyses from our cohort [74] assessed knee joint BMLs at four sites (medial tibial, media femoral, lateral tibial, and lateral femoral sites). This showed that 43% of participants had BMLs, of these 25% decreased in size and 24% increased in 2.7-year follow-up. In a younger cohort, Foong. et al followed 198 patients over eight years and found that knee BML size remained stable in over half of the participants [206]. However, both of these studies reported BMLs in the tibiofemoral compartment or total knee BMLs, and did not report patellar BMLs. The patellofemoral joint (PFJ) is a common site of pain [207] and contributes to functional limitation among OA patients [208]; thus, it is important to assess patellar sources of knee pain. Despite the high prevalence of PFJ OA, there are very few clinical or epidemiological studies that investigate PFJ OA particularly and the natural history of PFJ BMLs had not yet been described.

The aim of this study was, therefore, to describe the natural history of MRI-detected PFJ BMLs over 2.6 years and evaluated the association between increases in PFJ BMLs, knee pain and knee cartilage morphology in older adults.

## 4.2 Materials and Methods

### 4.2.1 Subjects

This study was performed as part of the Tasmanian Older Adult Cohort (TASOAC) study, a population-based study that was designed to identify the genetic, environmental, and biochemical factors associated with the development and progression of OA at multiple sites. Subjects between 50 and 80 years old were randomly selected from the electoral roll in Southern Tasmania (population 229,000), with an equal number of males and females. Baseline examinations were first taken in 2002 and follow-up measures were taken at approximately 2.6 years later. This study was approved by the Southern Tasmanian Health and Medical Human Research Ethics Committee. Written informed consent was obtained from all subjects.

### 4.2.2 Anthropometrics

Weight was measured with shoes, socks and bulky clothing removed using electronic scales (nearest 0.1kg). Height was measured with shoes, socks and headgear removed using a stadiometer (nearest 0.1cm). Body mass index (BMI) was calculated using height and weight ($kg/m^2$).

### 4.2.3 Magnetic Resonance Imaging

MRI scans of the right knees were performed on two occasions and imaged in the sagittal plane on a 1.5-T whole body magnetic resonance unit (Picker, Cleveland, OH) using a commercial transmit-receive extremity coil. The image sequences used are listed as follows: (1) a T1-weighted fat saturation 3D gradient recall acquisition in the steady state; flip angle 30°; repetition time 31 ms; echo time 6.71 ms; field of view 16 cm; 60 partitions; 512×512 matrix; acquisition time 11 min 56 s; one acquisition. Sagittal images were obtained at a partition thickness of 1.5 mm and an in-plane resolution of 0.31×0.31 (512×512 pixels). (2) a T2-weighted fat saturation 3-D fast spin echo, flip angle 90, repetition time 3067 ms, echo time 112 ms, field of view 16 cm, 15 partitions, 228x256-pixel matrix; sagittal images were obtained at a partition thickness of 4 mm with a between-slices gap of 0.5 to 1.0 mm. The image database was

transferred to an independent computer workstation using the software program Osirix (University of Geneva, Geneva, Switzerland) as previously described [116, 191].

### 4.2.4 Subchondral BML evaluation

Subchondral BMLs were measured by one observer (ZZ) trained by readers with long experience in scoring knee MRIs (CD) on T2-weighted MR images and were defined as increased signal areas adjoining to the subchondral bone at the patellar and trochlear sites of knees. The MRI slice with the greatest BML size were selected and scored using Whole-Organ Magnetic Resonance Imaging Score (WORMS) method [125]. Baseline and follow-up MRIs were scored in pairs in chronological order to minimise measurement error [74] (Figure 1). PFJ compartment BMLs were obtained by summing the BML scores of patella and trochlea (range 0-6). Each BML was scored according to the maximal percentage of bone area that the lesion occupied on the slice. We scored grade 0 if no bone marrow lesions were present; grade 1 if lesion size ≤25% of the region on the same slice; grade 2, 25% to 50% of the region on the same slice; grade 3, >50% of the region on the same slice [125]. The inter-rater reliability of this BML scoring system is excellent, as reported previously [71]. The intraclass correlation coefficient (ICC) was 0.94 for intra-observer repeatability. An increase of 1 or more grade on the 0 to 3-point scale from baseline to follow-up in BMLs was defined as an increase in BML. Those whose scores remained the same or decreased by 1 or more grade were regarded as stable or having regressed.

### 4.2.5 Cartilage defects assessment

Cartilage defects were assessed by a trained observer (CD) on T1-weighted MR image according to previously described criteria [126, 192]: grade 0 = normal cartilage; grade 1 = focal blistering and intracartilaginous low-signal intensity area with an intact surface and bottom; grade 2 = irregularities on the surface or bottom and loss of thickness less than 50%; grade 3 = deep ulceration with loss of thickness more than 50%; grade 4 = full-thickness chondral wear with exposure of subchondral bone. The worst grade was used if more than one defects existed at one site. Medial tibial, lateral tibial, medial femoral, lateral femoral, trochlear and patellar compartments were

measured. Scores for patellofemoral and tibiofemoral compartment were obtained by summing the individual defect scores in relevant regions. Intraclass correlation coefficients (ICCs) ranged from 0.80 to 0.95 for intraobserver reliability [209].

### 4.2.6 Cartilage volume measurement

Knee tibial and patellar cartilage volumes were measured on T1-weighted MR images by a single trained observer at baseline as previously described [126]. The volumes of individual cartilage plates (medial tibial, lateral tibial, medial femoral, lateral femoral, patellar and trochlear) were isolated from the total volume by manually drawing disarticulation contours around the cartilage boundaries on a section by section basis. These data were then resampled by means of bilinear and cubic interpolation (area of 312×312 um and 1.5 mm thickness, continuous sections) for the final 3-dimensional rendering. The volume of patellofemoral and tibiofemoral compartments were obtained by summing the pertinent cartilage plates within compartments. The coefficient of variation (CV) for cartilage volume measures was 2.1% for medial tibial, and 2.2% for lateral tibial, 2.7% for medial femoral, 2.8% for lateral femoral, 2.6% for trochlea and 2.6% for patella [194].

### 4.2.7 Meniscal damage

Meniscal damage was assessed by a trained observer on T1-weighted MR images as previously described [197]. The proportion of the menisci affected by a tear, partical or full extrusion was scored separately (yes/no) at the anterior, middle and posterior horns (medially/laterally). Anterior, middle and posterior scores were summed to get medial and lateral meniscal tear/extursion scores. The intra-and inter-observer correlation coefficient ranged from 0.86 to 0.96 for meniscal tear and 0.85 to 0.92 for meniscal extrusion [198].

### 4.2.8 WOMAC knee pain assessment

Knee pain was assessed using the Western Ontario McMaster Osteoarthritis Index (WOMAC) [204] at baseline, 2.6 and 5 years later using a 10-point scale from 0 (no pain) to 9 (most severe pain). The 5 subscales (walking on flat surface, going up/down

stairs, at night, sitting/lying and standing upright) were assessed separately and summed to create a total pain score (0 to 45).

### 4.2.9 Radiographic osteoarthritis

A standing anteroposterior semiflexed view of the right and left knee with 15°of fixed knee flexion was performed in all subjects at baseline and scored individually for osteophytes and joint space narrowing (JSN) on a scale of 0-3 (0=normal and 3=severe) according to the Altman atlas as previously described [189]. Medial tibiofemoral or lateral tibiofemoral JSN was scored separately, while osteophytes were scored at each site of medial tibia, medial femur, lateral tibia and lateral femur. The prevalence of medial or lateral tibiofemoral JSN or osteophytes was defined as the presence of radiographic osteoarthritis (ROA) [190].

### 4.2.10 Smoking

Smoking status (never, or ever) was determined by questionnaire from the following questions: “Have you ever smoked cigarettes on a regular basis?”

### 4.2.11 Knee bone size measurement

Knee tibial plateau bone areas were measured using the software program Osiris as previously described [116]. Medial and lateral tibial plateau bone areas were uniform in nature and can be measured directly from the reformatted axial images. The CVs for these measures are 2.2-2.6% [116, 189].

### 4.2.12 Data analysis

Student’s t-tests and $\chi^2$ tests were used to compare differences between subjects with and without an increase in PFJ BMLs. Crude and adjusted linear regression was used to assess whether PFJ BML changes over 2.6 years were associated with changes in knee pain in the different sub-scales over 5 years, before and after adjustment for potential confounders (age, sex, BMI, baseline patellar BML, ROA, smoking status). Crude and adjusted log binomial regression was used to examine the associations

between increases in PFJ BMLs as an outcome, and baseline cartilage volumes as well as baseline cartilage defect scores as predictors, both before and after adjustment for potential confounders. A *p*-value less than 0.05 (2-tailed) was considered statistically significant. All statistical analyses were performed on Stata version 12.0 for Windows (StataCorp, College Station, TX, USA).

## 4.3 Results

### 4.3.1 Characteristics of the study population

1100 participants aged between 51 and 81 (mean 63 years) were recruited to the TASOAC study. 406 subjects completed MRI measures at baseline and follow-up. MRI scans were discontinued after this sample due to decommissioning of the MRI scanner. WOMAC knee pain data were available on these subjects at 5 years' follow-up. As previously reported [210], study participants who did not complete MRI measures were similar to the remainder of the cohort in terms of demographics, smoking status, cartilage defects, BMLs, cartilage volume and ROA at baseline.

The characteristics of the study sample grouped by increase or no increase in PFJ BMLs at 2.6 years follow-up are presented in Table 1. Subjects who had an increase in PFJ BMLs had a higher patellar cartilage defect score and lower patellar cartilage volume at baseline and were less likely to have smoked but were similar in terms of BMI. There were no significant differences in age, sex, BMI, knee pain and ROA (%) between people with increased PFJ BMLs and those without (Table 1).

**Table 4. 1** Characteristics of the participants

| | Patellofemoral joint BMLs | | |
|---|---|---|---|
| | No Increase (n=321) | Increase (n=85) | *p* value |
| Age | 62.9±7.1 | 61.8±7.6 | 0.25 |
| Females (%) | 50 | 50 | 0.86 |
| BMI (kg/m$^2$ ) | 27.5±4.3 | 28.0±4.9 | 0.38 |
| ROA present (%) | 70 | 61 | 0.75 |
| Knee pain (%) | 50 | 40 | 0.54 |
| Ever smoked (%) | 50 | 40 | **0.04** |
| Tibia Bone size (cm$^2$) | 33.6±4.9 | 32.7±4.2 | 0.13 |
| PFJ BMLs at baseline (%) | 26 | 30 | 0.38 |
| TFJ BMLs at baseline (%) | 32 | 42 | 0.09 |
| PFJ Cartilage defect score at baseline | 3.2±1.5 | 4.0±1.6 | **<0.01** |
| TFJ Cartilage defect score at baseline | 4.2±1.8 | 4.4±1.6 | 0.37 |
| PFJ cartilage volume at baseline (ml) | 6.5±1.6 | 6.2±1.5 | 0.07 |
| TFJ cartilage volume at baseline (ml) | 10.4±2.6 | 10.1±2.1 | 0.29 |

Abbreviation: ROA, radiographic osteoarthritis; BMI, body mass index; PFJ, patellofemoral joint; TFJ, tibiofemoral joint; BMLs, bone marrow lesions. *Data are given as mean ± SD unless otherwise indicated. Student's t-test or chi-square test (where appropriate) were used to test for significant differences between two groups. An increase in patellofemoral joint (PFJ) BMLs is defined as a change in BMLs of ≥1 from baseline to follow-up (vs. no increase).

### 4.3.2 Natural history of PFJ BMLs

109 of 406 participants (27%) who completed follow-up had PFJ BMLs at baseline. Of these participants, 49 (45%) persisted, 26 (24%) increased in grade, and 34 (31%) improved including 23 (21%) which completely resolved. PFJ BMLs were absent in a total of 297 subjects at baseline, of which 59 developed new BMLs (19.7% of knees) over 2.6 years (Figure 4.1, Figure 4.2).

Grade 1 BMLs (≤25% of the region on the same slice) changed most often, accounting for the majority of newly induced lesions (76.3%), and lesions that resolved over 2.6 years (87%).

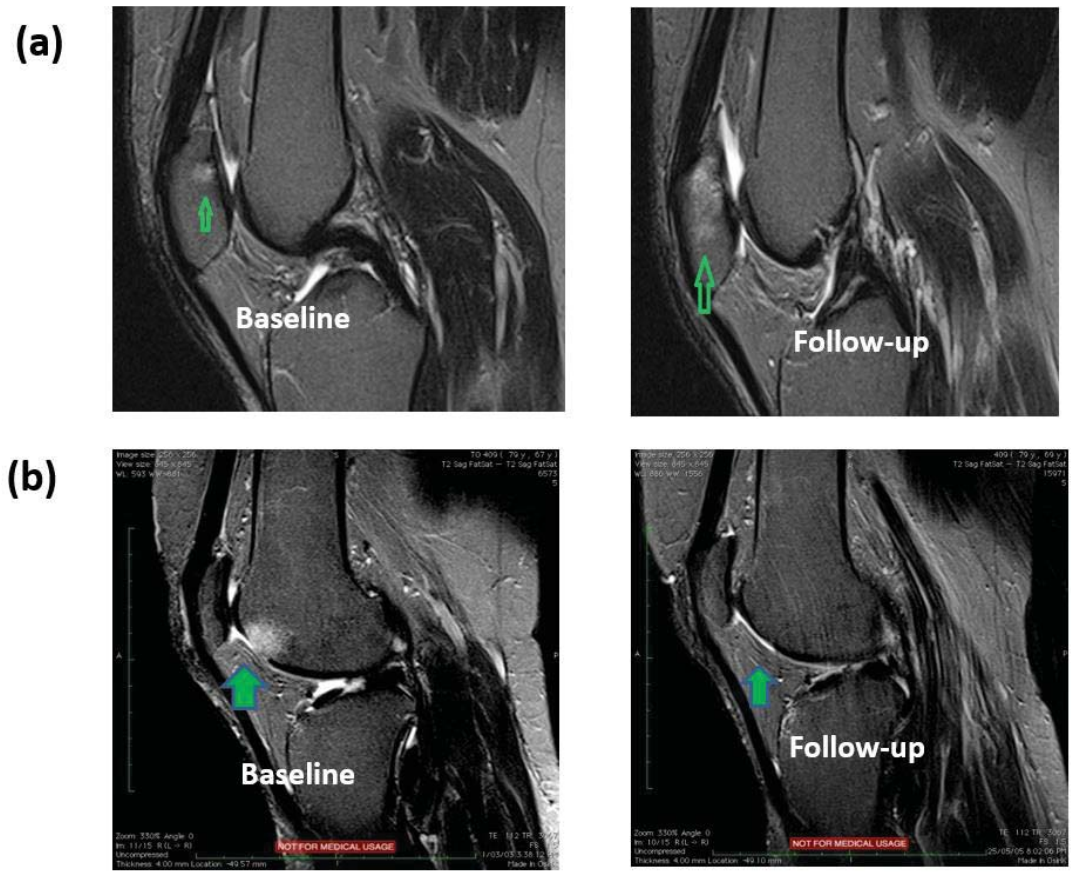



**Figure 4. 1** Examples of change in BMLs over 2.6 years

(a) PFJ BMLs increases from baseline to follow-up. (b) PFJ BMLs completely resolved from baseline to follow-up.

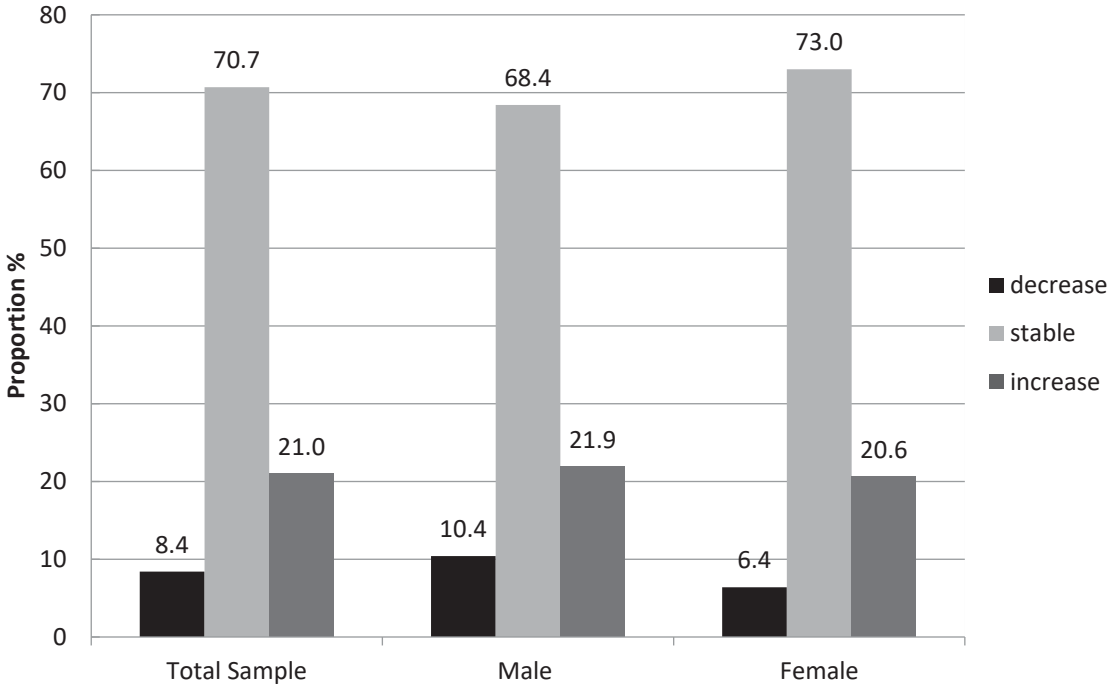



**Figure 4. 2** Natural history of patellofemoral bone marrow lesions over 2.6 years in older adults

### 4.3.3 WOMAC pain and PFJ BMLs

Table 2 describes the association between changes in PFJ BMLs over 2.6 years and changes in WOMAC knee pain over 5 years. A change in PFJ BML score over 2.6 years was associated with a change in total WOMAC pain and knee pain when going up and down stairs over 5 years, after adjustment for age, sex, BMI, ROA, smoking status, baseline PFJ BMLs and baseline cartilage defects. No statistically significant associations between PFJ BMLs changes and changes in other WOMAC knee pain subscales were observed.

**Table 4. 2** Associations between change in PFJ BMLs over 2.6 years and changes in WOMAC pain over 5 years

| | Univariable β (95% CI) | Multivariable * β (95% CI) | Multivariable** β (95% CI) |
|---|---|---|---|
| Total WOMAC knee pain | **0.78 (0.15, 1.40)** | **0.81(0.17, 1.45)** | **0.67 (0.03, 1.31)** |
| Pain walking on a flat surface | 0.03 (-0.12, 0.18) | 0.03 (-0.12, 0.19) | 0.02 (-0.14, 0.18) |
| Pain going up and down stairs | **0.28 (0.07, 0.48)** | **0.27 (0.07, 0.47)** | **0.24 (0.04, 0.44)** |
| Pain at night when in bed | **0.22 (0.03, 0.41)** | **0.22 (0.02, 0.42)** | 0.17 (-0.03, 0.37) |
| Pain sitting or lying | 0.12 (-0.02, 0.27) | 0.13 (-0.02, 0.28) | 0.11 (-0.05, 0.26) |
| Pain standing upright | 0.13 (-0.02, 0.29) | 0.15 (-0.01, 0.32) | 0.15 (-0.03, 0.29) |

*Adjusted for age, sex, BMI, ROAand smoking status, **further adjusted for baseline patellofemoral BML, baseline tibiofemoral BMLs, baseline patella cartilage defect, baseline tibiofemoral cartilage defect and baseline knee pain. Bold denotes statistical significance.

### 4.3.4 Cartilage morphologies and PFJ BMLs

Table 4.3 describes the relationship between baseline cartilage volume, baseline cartilage defects, and increases in PFJ BMLs over 2.6 years. Higher PFJ and TFJ cartilage volume at baseline was associated with reduced risks of having an increase in PFJ BMLs. This result remained significant after adjustment for age, sex, BMI, smoking status, ROA, baseline patellar BMLs, and baseline total cartilage defects, total cartilage volume, meniscal tears and meniscal extrusion. Baseline PFJ cartilage defects were associated with increased odds of having an increase in PFJ BMLs; this association also remained significant after adjustment for age, sex, BMI, ROA, smoking status and baseline patellar BMLs and further adjustment for baseline PFJ BMLs, baseline total tibiofemoral BMLs, total cartilage defects, total cartilage volume, meniscal tears and meniscal extrusion. There were no significant associations between cartilage defects at the tibiofemoral compartment and increases in PFJ BMLs.

Figure 4.3 shows the association between baseline PFJ cartilage defects and increase in PFJ BMLs. There was a higher rate of increased PFJ BMLs in those with higher baseline PFJ cartilage defects scores especially grade 3 and 4.

**Table 4. 3** Associations between baseline cartilage volume, baseline cartilage defects, and increases in PFJ BMLs over 2.6 years

| | Univariable | Multivariable * | Multivariable ** |
|---|---|---|---|
| | RR (95% CI) | RR (95% CI) | RR (95% CI) |
| Baseline PFJ cartilage volume (ml) | **0.88 (0.77, 0.99)** | **0.71 (0.57, 0.87)** | **0.69 (0.52, 0.90)** |
| Baseline TFJ cartilage volume (ml) | 0.96 (0.89, 1.03) | **0.85 (0.74, 0.97)** | **0.67 (0.49, 0.92)** |
| Baseline PFJ cartilage defects (per grade) | **1.48 (1.24, 1.76)** | **1.46 (1.22, 1.76)** | **1.73 (1.38, 2.17)** |
| Baseline TFJ cartilage defects (per grade) | 1.04 (0.96, 1.13) | 1.03 (0.93, 1.14) | 0.99 (0.89, 1.10) |

PFJ, patellofemoral joint; TFJ, tibiofemoral joint; *Adjusted for age, sex, BMI, baseline patella BMLs, smoking status and ROA. ** further adjusted for baseline PFJ BMLs, baseline total tibiofemoral BMLs, total cartilage defects and total cartilage volume, meniscal extrusion and meniscal tears. Bold denotes statistical significance.

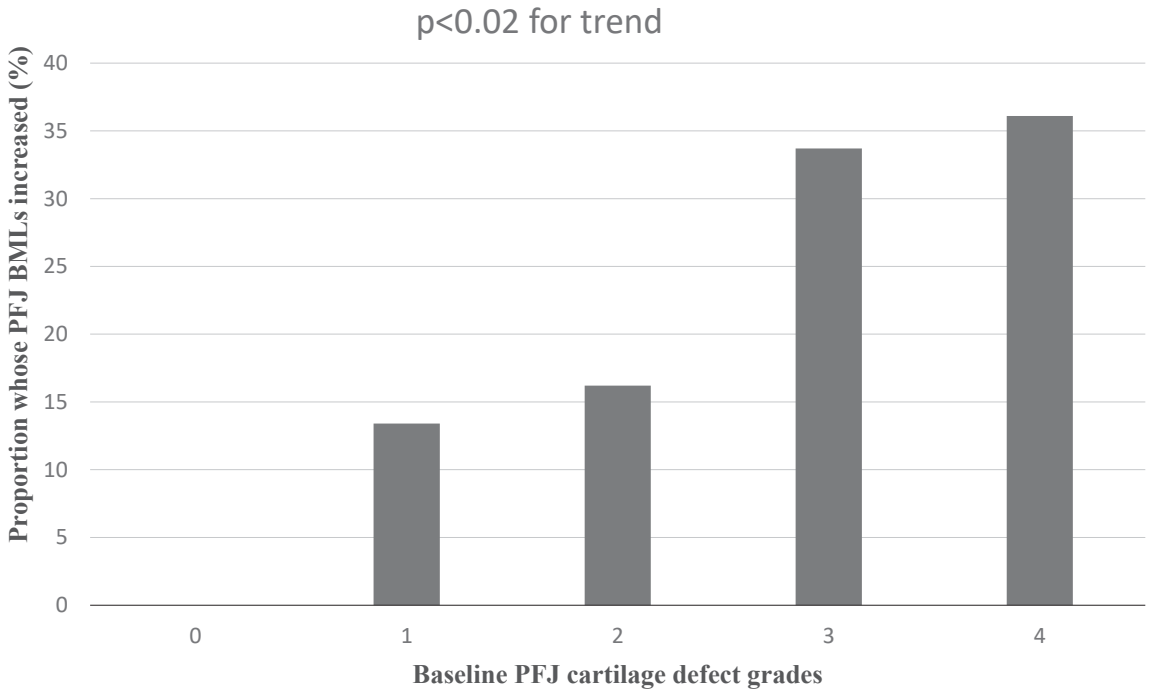



**Figure 4. 3** Association between PFJ cartilage defect grades at baseline and increases in PFJ BMLs

## 4.4 Discussion

This is the first population-based longitudinal study that describes the natural history of PFJ BMLs and the associations with WOMAC knee pain as well as knee structures. PFJ BMLs were not static, with over one third participants showing change (both increasing and decreasing) over 2.6 years of follow-up. Changes in PFJ BMLs over 2.6 years were associated with change in knee pain when going up or down stairs over 5 years, suggesting that changes in PFJ BMLs are clinically relevant. Furthermore, baseline PFJ cartilage volume was associated with a decrease in PFJ BMLs and baseline PFJ cartilage defects were associated with an increase in PFJ BMLs, indicating a site-specific effect of cartilage morphology on PFJ BMLs.

We found that approximately half of the pre-existing PFJ BMLs showed a change in size over 2.6 years, with one third of pre-existing PFJ BMLs decreasing in score over 2.6 years. The regression rate was higher than some previous studies which assessed BMLs in whole knee [85, 211], but lower than that in a study which described knee joints BMLs in a cohort with symptomatic knee OA over 30 months [72]. The majority of the subjects whose BMLs resolved (87%) and those with new BMLs (76%) had grade 1 PFJ BMLs, suggesting that milder PFJ BMLs when BMLs have a greater potential to reverse.

Many studies have linked TF BMLs and knee pain [75, 83, 84, 212]. Our team previously reported that tibiofemoral BMLs were associated with knee pain [74] and patellar BMLs were deleteriously associated with increased knee pain especially going up/down stairs [195]. However, associations between knee pain and PFJ BMLs has been less frequently reported [44, 213, 214]. The current study found that the change in PFJ BMLs over 2.6 years was associated with changes in total WOMAC pain score and knee pain when going up and down stairs over 5 years. This model was not same time frame but does allow assessment of whether changes in PFJ BMLs over 2.6 years predict changes in WOMAC knee pain over 5 years. The results were expected given that PFJ structural changes are commonly thought to be the causes of anterior knee pain during activities in which the knee is flexed, such as squatting and going up and down stairs [215].

Previous studies assessing the whole knee suggest that subchondral bone might play a role in cartilage degradation and in the early development of knee clinical symptoms [216], with subchondral BMLs predicting increased progression of cartilage defects and loss of cartilage volume [79, 85]. However, change in BMLs may be secondary to the cartilage damage. This finding was in line with our previous finding that baseline cartilage defects predicted BML progression in the tibiofemoral compartment [205]. In order to confirm this hypothesis in the PFJ, we examined whether baseline cartilage morphology could predict increases in PFJ BMLs. Baseline PFJ cartilage volume was associated with a decrease in PFJ BMLs and baseline PFJ cartilage defects were associated with an increase in PFJ BMLs over 2.6 years. Meniscal pathology has been reported to have a substantially increased risk of both incident and enlarging BML over 30 months [217], however, in our current study, significant associations between cartilage morphology and increases in PFJ BMLs remained unchanged after further adjustment for meniscal tears and meniscal extrusion, indicating independent associations between PFJ cartilage morphology and PFJ BMLs. The current study also found that tibiofemoral cartilage defects were not associated with changes in PFJ BMLs, suggesting compartment specific associations. This is consistent with the tibiofemoral compartment [61, 205]. Smoking has been found to be related to knee BMLs [218] as well as knee pain and cartilage defects [219, 220]. So it was considered as a potential confounder. However, no significant differences were made after smoking status was adjusted in multivariable analyses.

There are several potential limitations in this study. First, follow-up MRI scans were only available for the current study in 406 out of 1100 participants. However, between the current study sample and the rest of cohort were similar in terms of demographics, ROA, baseline cartilage volume, defects and BMLs (data not shown). Secondly, BML scores were measured by grading the slice with the greatest BML size using a semi-quantitative scale (0 to 3) rather than a quantitative measure of area or volume, which is likely to be more sensitive. Considering the slice thickness (4 mm) and interslice gap (0.5 to 1.0mm) of our imaging protocol, some lesions (especially small ones) may be underestimated if most of them lies within the interslice gap. Thirdly, we were not able to measure knee alignment, which has been linked to BMLs [221, 222]. However, it remains uncertain whether this is a cause effect association. Fourth, patella axial and

lateral firm were not included in the radiographic examination, which may impair the veracity of ROA assessment.

In conclusion, PFJ BMLs are not static and change is clinically relevant. PFJ cartilage morphology predicts increases in PFJ BMLs

# Chapter 5:

# Associations between serum levels of hs-CRP, knee bone marrow lesions and knee pain in patients with knee osteoarthritis

This manuscript has been published (Zhu *et al* Arthritis Care & Research. 2016; 68(10)). The typeset version of the manuscript as it appeared in the journal is in Appendix 2. The text of this chapter is the same as the published version, except where changes have been requested by the examiners. Thus, there is some repetition of the methods.

## 5.1 Introduction

OA is a common joint disease characterised by progressive deterioration of the entire joint [223]. Although OA has traditionally been regarded as a non-inflammatory arthritis, recent studies have suggested that systemic inflammation is involved in its pathogenesis [26, 152], albeit at much lower levels than the recognised inflammatory arthropathies. Serum levels of interleukin (IL)-6, an inflammatory cytokine, predicts radiographic knee OA progression over 5-10 years in women [143] and increased knee cartilage volume loss over 3 years in older adults [142]. Similarly, increased serum levels of inflammatory cytokines IL-1β and tumour necrosis factor (TNF)-α are associated with increased risk of knee OA progression [10].

High-sensitivity C-reactive protein (hs-CRP) is a circulating marker of systemic inflammation. It is synthesized by the liver and can be up-regulated by IL-6 and IL-1 [174] and is associated with presence and progression of knee OA [177, 179]. A recent meta-analysis found that serum hs-CRP levels in people with OA were modestly but significantly higher than in controls, and were weakly but significantly associated with knee pain and decreased physical function [224]. However, this analysis was based on five cross-sectional studies, so whether these associations are causal is unknown [224]. We reported that serum hs-CRP was associated with increased knee pain over 5 years in older adults [225].

Bone marrow lesions (BMLs) are ill-defined hyperintense signals seen on magnetic resonance imaging (MRI) scans. They are recognized as an important feature of knee OA and play a vital role in disease progression [78, 79]. BMLs are related to clinical manifestations [76] and radiographic abnormalities in OA patients [61, 85].

Histologically, BMLs represent multiple abnormalities including osteonecrosis, trabecular abnormalities and bone remodelling, but rarely edema [226]. BMLs may also be caused by inflammation in reaction to cartilage breakdown products, or other factors in intruded synovial fluid [87]. However, thus far, it is unclear if inflammation is associated with BMLs in OA patients. BMLs could be an intermediate factor on the

causal pathway between inflammatory markers and knee pain, but it is not known if serum levels of hs-CRP are associated with knee BMLs in patients with knee OA.

Therefore, the aims of this study were to describe the associations between serum hs-CRP, knee BMLs and knee pain cross-sectionally and longitudinally in patients with knee OA.

## 5.2 Patients and methods

### 5.2.1 Study design and patient population.

These data were collected for an observational sub-study from the Vitamin D Effects on Osteoarthritis (VIDEO) Study, which was a multicentre, parallel randomized, placebo-controlled and double-blind clinical trial in patients with symptomatic knee OA and a low 25-hydroxyvitamin D (25OHD) level in Melbourne and Hobart, Australia. The first 192 patients (mean 63 years, range 49-79 years, female 53%) in Hobart were selected from the wider VIDEO study population to have hs-CRP measurement. Inclusion and exclusion criteria were the same as for the VIDEO study [202]. Eligible subjects were assessed according to the American College of Rheumatology (ACR) criteria for clinical knee OA [6] with a pain score of at least 20 mm on a 100 mm visual analogue scale (VAS). They also had an ACR function class rating of I, II and III [199], and relatively good health, with a score of 0 to 2 on a 5 point Likert scale (from 0 indicating very good health to 4 indicating very poor health) according to the global investigator assessment of disease status [227].

Subjects were included if their serum 25OHD levels >12.5 nmol/L and < 60 nmol/L. Exclusion criteria included grade 3 radiographic changes according to Altman's atlas [31] severe knee pain on standing (more than 80 mm on a 100 mm VAS), contraindication to MRI, rheumatoid or psoriatic arthritis, lupus, cancer, and severe cardiac or renal impairment.

The VIDEO study was approved by The Tasmania Health and Human Medical Research Ethics Committee (reference number H1040) and Monash University Human

Research Ethics Committee (reference number CF10/1182-2010000616). Written informed consent was obtained from all participants.

### 5.2.2 Serum hs-CRP measurements

Serum levels of hs-CRP were measured at baseline and after 24 months using enzyme-linked immunosorbent assay [203]. The minimal detectable concentration is approximately 0.02 μg/ml. The coefficients of variation (CVs) in our hands was 5.8%-6.3%. Change in serum levels of hs-CRP was calculated as: follow-up value − baseline value.

### 5.2.3 Assessment of bone marrow lesions

BMLs in the diseased knee (or of the less painful knee if both knees were affected to avoid "ceiling effect") were assessed on MR images, acquired with a 1.5T whole-body magnetic resonance unit (Picker, Cleveland, Ohio, USA) using a commercial transmit–receive extremity coil at baseline and 24 months later. T2-weighted fast spin echo (FSE) sequences were used, as previously described [202].

BMLs were measured by one trained observer (ZZ) using a modified whole organ MRI score (WORMS) [125]. BMLs were defined as ill-defined hyperintense signal areas adjoining to the subchondral bone at 6 regions: medial patellar, lateral patellar, medial tibial, lateral tibial, medial femoral and lateral femoral.

In the modified WORMS, the medial and lateral tibial and femoral compartments are divided into three sub-regions (anterior, central, posterior), and the tibia has an additional subspinous sub-region which represents the portion of the tibia beneath the tibial spines. Thus, a total of 15 sub-regions were scored for each knee [125].

BMLs were scored categorically according to the maximal percentage of bone area that the lesion occupied within the total sub-region. We scored grade 0 if no BMLs; grade 1 if lesion size =<25% of the subregion; grade 2, 25% to 50% of the subregion; grade 3, >50% of the subregion [125]. Total knee BML scores were obtained by summing the

BML scores of all the sites, giving a potential total knee BML score range of 0 to 45. Baseline and follow-up MRIs were scored in pairs in chronological order to minimise measurement error [74]. The intraclass correlation coefficients (ICCs) were 0.93-0.98 for intra-observer repeatability.

Presence of BMLs in the whole knee was defined as a BML score of ≥ 1 at any subregion. Changes in BML score were calculated by subtracting total knee BMLs at follow-up from total knee BMLs at baseline. An increase in BML was defined as a change in BML score of ≥ 1.

### 5.2.4 WOMAC knee pain assessment

Knee pain was assessed at knee level using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) [204] at baseline and 24 months later. Five items were used to assess pain: walking on flat surface, when going up/down stairs, at night in the bed, when sitting/lying and when standing upright; these were assessed using 100 mm visual analogue scales. Items were summed to create a total pain score (range 0-500). Change in total knee pain was calculated as (follow-up value – baseline value of total pain scores).

### 5.2.5 Anthropometrics

Height was measured to the nearest 0.1 cm (with shoes, socks, and headgear removed) using a stadiometer. Weight was measured to the nearest 0.1 kg (with shoes, socks, and bulky clothing removed) using a single pair of electronic scales (Model 707; Seca Delta, Hamburg, Germany) that were calibrated using a known weight at the beginning of each clinic. Body mass index (BMI) [weight (kg)/height ($m^2$)] was calculated.

### 5.2.6 Co-morbidities

Co-morbidities (no or yes) including diabetes, hypertension, osteoporosis, asthma, bronchitis, emphysema, heart attack, stroke, angina and high cholesterol were recorded using the following questionnaire: have you ever been told by your doctor or a nurse that you have any of the following conditions?

### 5.2.7 25OHD assays

In the original RCT study, after randomisation, participants in the intervention and control arms were given a monthly capsule of 50,000 IU (1.25 mg) vitamin D3 (cholecalciferol) and placebo, respectively, for 24 months. Serum 25OHD was assayed by Liaison method at screening and month 24, utilizing a direct competitive chemiluminescent immunoassays (DiaSorin Inc, Stillwater, Minnesota, USA). The intra-assay and interassay coefficients of variation were 3.2% and 6.0%.

### 5.2.8 Data analysis

T tests or $\chi^2$ tests (when appropriate) were used to compare means or proportions between those with higher (≥ median value) and lower (<median value) hs-CRP. Baseline hs-CRP was not normally distributed; hence quartiles of baseline hs-CRP were used for subsequent analyses as no other transformations were appropriate. Univariable and multivariable log binomial regression analysis was used to determine associations between baseline hs-CRP and presence of knee BMLs in the whole knee and an increase in total knee BMLs before and after adjustment for age, sex, BMI, intervention arm (vitamin D or control, for longitudinal data), and/or baseline total knee BML scores (for longitudinal data). Linear regression analyses were used to assess associations between changes in hs-CRP and changes in total knee BMLs, and between baseline/changes in hs-CRP and baseline/changes in total WOMAC knee pain before and after adjustment for covariates including interventions. Associations between baseline/changes in hs-CRP and baseline/changes in total WOMAC knee pain were further adjusted for baseline total knee BMLs or changes in BML score to see if these associations were explained by BMLs.

A *p* value less than 0.05 (two tailed) was regarded as statistically significant. All statistical analyses were performed on Stata version 12.0 for Windows (StataCorp, College Station, TX, USA).

## 5.3 Results

### 5.3.1 Characteristics of participants

A total of 192 subjects (53% female) aged between 49 and 79 years old (mean 63 years) were included in the study. The median serum level of hs-CRP was 1.40 µg/ml (range 0.18 – 27.8 µg/ml). The average vitamin D level of participants at baseline and month 24 were 45 ± 14 nmol/L and 70 ± 25 nmol/L, respectively. Increases in vitamin D levels were mainly attributed to intervention (vitamin D supplement on intervention arm). BMLs were present in 80% of subjects. Those who had higher levels of hs-CRP (≥ median level) were heavier, shorter and had greater BMI as well as higher knee pain scores than those with lower hs-CRP levels (< median level). No significant differences were found between groups in terms of sex, baseline total knee BML scores and baseline 25OHD levels (Table 1). In total, 108 participants had bilateral symptomatic knee OA. There were no significant differences in terms of hs-CRP levels and pain score between subjects with bilateral and unilateral knee OA (data not shown). There were no significant interactions between knee OA status (bilateral or unilateral knee OA) and hs-CRP on increase/change in BMLs (all p>0.40), so patients with bilateral and unilateral knee OA were combined for analyses.

**Table 5. 1** Characteristics of the participants at baseline

| | Baseline hs-CRP < median (n=96) | Baseline hs-CRP ≥ median (n=96) | *p* |
|---|---|---|---|
| Age, years | 63.0 (7.7) | 63.2 (7.1) | 0.85 |
| Women (%) | 58% | 49% | 0.20 |
| Weight | 84.0 (14.0) | 88.4 (15.5) | **<0.001** |
| Height | 170.9 (10.5) | 168.1 (8.8) | **0.04** |
| BMI, kg/m$^2$ | 27.7 (4.2) | 31.3 (4.8) | **<0.001** |
| 25OHD, nmol/L | 45.2 (14.4) | 44.2 (12.8) | 0.62 |
| Total knee BML score | 3.14 (3.1) | 3.99 (3.5) | 0.08 |
| Total WOMAC score | 103.4 (82.4) | 141.3 (78.3) | **0.001** |
| Prevalence of BMLs | 76.3% | 85.0% | 0.12 |

Results are shown as mean (SD) except for percentage. Two-tailed t test used for differences between means, $\chi^2$ test used for proportions (percentages). Significant differences are shown in bold. hs-CRP, high-sensitivity C-reactive protein; BMI, body mass index; BML, bone marrow lesion, 25OHD: 25-

hydroxyvitamin D; WOMAC: Western Ontario McMaster Osteoarthritis Index. Median level of serum hs-CRP: 1.4 µg/ml.

### 5.3.2 Serum hs-CRP and total knee BMLs

In cross-sectional analysis, quartiles of baseline hs-CRP were positively associated with the presence of knee BMLs, before and after adjustment for age, sex and BMI (Figure 1a, Table 2). In longitudinal analysis, quartiles of baseline hs-CRP were significantly and positively associated with an increase in total knee BMLs (Figure 1b, Table 2). This result remained significant after adjustment for age, sex, BMI, interventions and baseline total knee BML scores (Table 2). Significant associations were found between change in hs-CRP and changes in total knee BML scores, both before and after adjustment for potential confounders (Table 2). Additionally, the highest quartile of hs-CRP (cut-off point: 2.6 µg/ml) was associated with an increase in total BMLs in multivariable analyses (the highest vs other quartiles: RR=1.86, 95%CI: 0.98, 3.57; the highest vs the lowest quartile: RR=1.42, 95%CI: 1.01, 1.99).

**Table 5. 2** Associations between serum hs-CRP and total knee BMLs: cross-sectional & longitudinal data

| | Univariable | Multivariable* |
|---|---|---|
| **Presence of total knee BMLs** | PR (95%CI) | PR (95%CI) |
| Baseline hs-CRP (per quartile) | **1.06 (1.00, 1.14)** | **1.07 (1.00, 1.15)** |
| **Increase in total knee BMLs** | RR (95%CI) | RR (95%CI) |
| Baseline hs-CRP (per quartile) | **1.22 (1.01, 1.47)** | **1.37 (1.10, 1.70)** |
| **Change in total knee BMLs** | β (95%CI) | β (95%CI) |
| Change in hs-CRP (per µg/ml) | **0.18 (0.03, 0.33)** | **0.19 (0.05, 0.34)** |

*Adjusted for age, sex, BMI, interventions (for longitudinal data), and/or baseline total knee BMLs (for longitudinal data). Statistical significances are shown in bold. Hs-CRP, high-sensitivity C-reactive protein; BMLs, bone marrow lesions; PR, prevalence ratio; RR: risk ratio.

### 5.3.3 Serum hs-CRP and total WOMAC knee pain

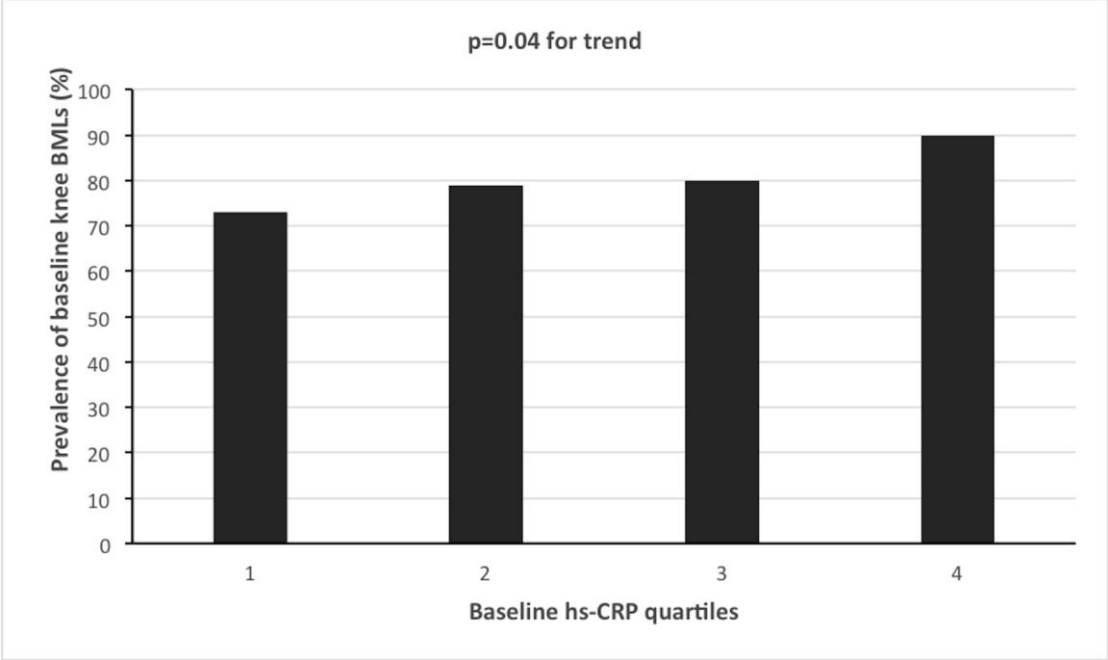
In cross-sectional analyses, quartiles of baseline hs-CRP were associated with total knee pain in unadjusted analysis (Figure 2a). The association remained statistically significant after adjustment for age, sex and BMI, and did not change after further adjustment for baseline total knee BML score (Table 3). In longitudinal analyses, baseline hs-CRP was not significantly associated with change in total knee pain, before or after adjustment for age, sex, BMI, interventions and baseline knee pain scores (Table 3); however, change in hs-CRP was significantly associated with change in total knee pain before and after adjustment for covariates (Table 3). This association decreased in magnitude and was no longer significant after further adjustment for change in total knee BMLs (Table 3). Additionally, quartiles of change in hs-CRP were significantly associated with an increase in total knee pain (Figure 2b).

The significant associations between hs-CRP and knee BMLs/pain remained unchanged after further adjustment for the co-morbidities (data not shown).

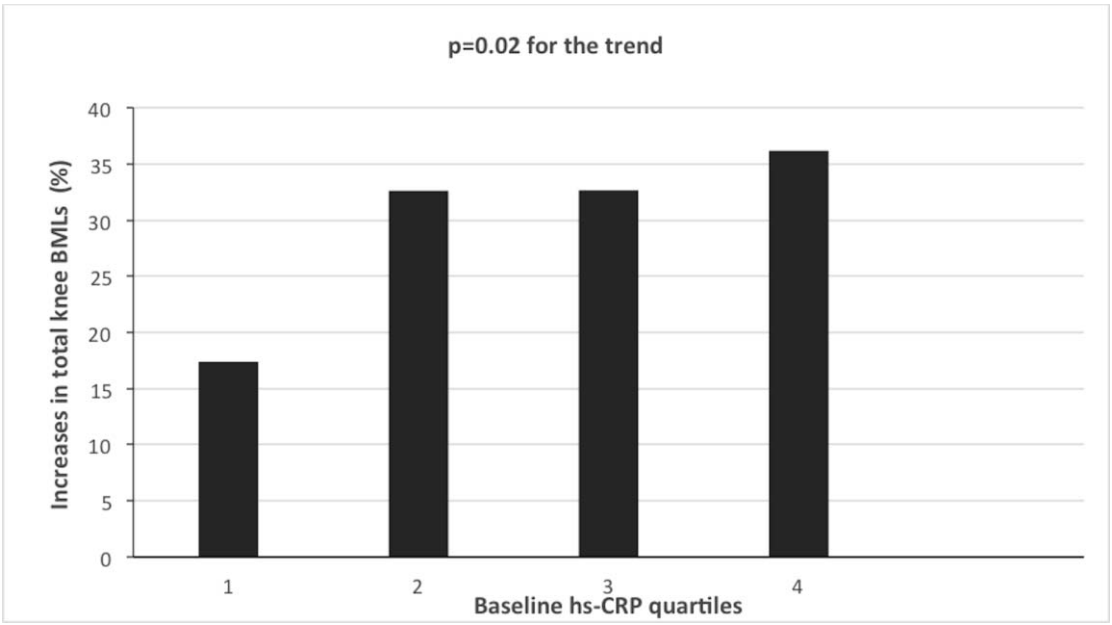
**Table 5. 3** Associations between serum hs-CRP and total WOMAC knee pain: cross-sectional & longitudinal data

| | Multivariable* β (95%CI) | Multivariable** β (95%CI) |
|---|---|---|
| **Total knee pain at baseline** | | |
| Baseline hs-CRP (per quartile) | **13.8 (2.46, 25.2)** | **13.7 (2.26, 25.1)** |
| **Change in total knee pain** | | |
| Baseline hs-CRP (per quartile) | 5.21 (-6.48, 16.9) | 5.16 (-6.56, 16.9) |
| Change hs-CRP (per µg/ml) | **4.71 (0.48, 8.94)** | 4.24 (-0.24, 8.51) |

*Adjusted for age, sex, BMI, interventions (longitudinal), and/or baseline knee pain (for knee pain change); ** Further adjustment for baseline total knee BMLs (cross-sectional/or longitudinal), or change in total knee BMLs (longitudinal). Statistically significant associations are shown in bold. Hs-CRP, high-sensitivity C-reactive protein; BMLs, bone marrow lesions; WOMAC: Western Ontario McMaster Osteoarthritis Index.


p=0.04 for trend
Prevalence of baseline knee BMLs (%)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
Baseline hs-CRP quartiles

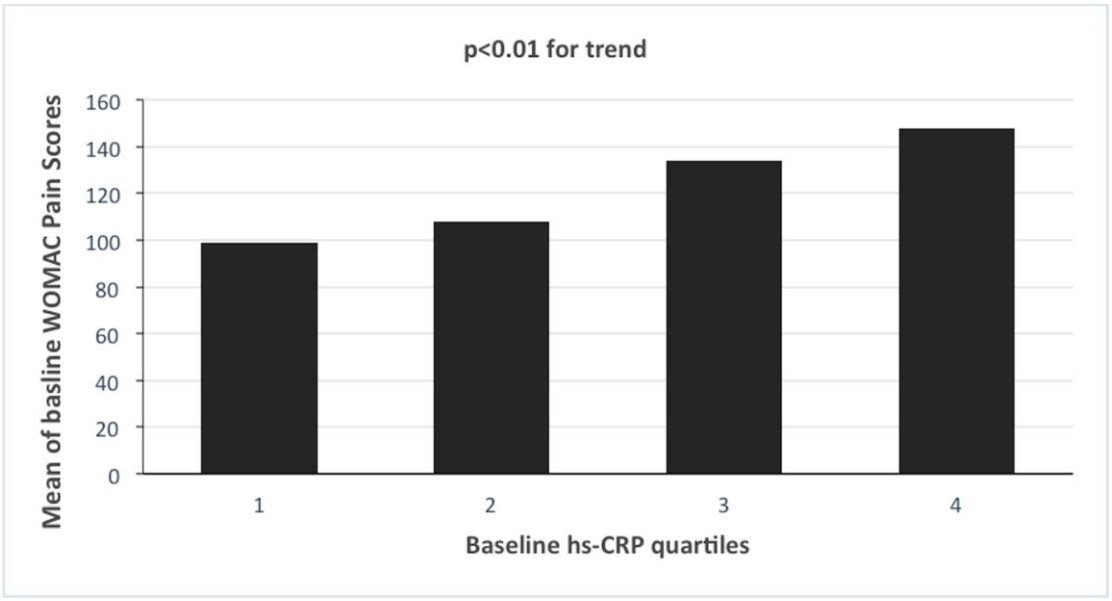
(**a**)



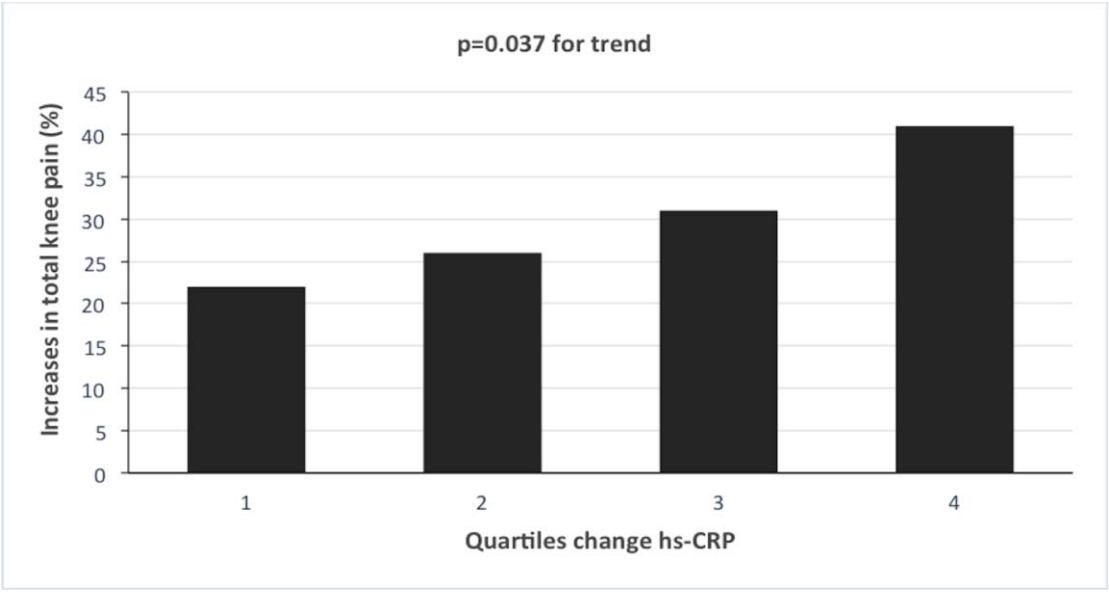

(**b**)

**Figure 5. 1** Association between hs-CRP and knee BMLs

(a) Baseline prevalence of knee BMLs, by quartiles of hs-CRP: (b) Presence of an increase in total knee BMLs over 2 years by baseline hs-CRP (quartile). Hs-CRP: high sensitivity C-reactive protein; BMLs: bone marrow lesions




(**a**)




(**b**)

**Figure 5. 2** Associations between hs-CRP and knee pain

(a) Baseline WOMAC knee pain scores by baseline hs-CRP (quartiles). (b) Presence of an increase in total WOMAC knee pain by change in hs-CRP (quartiles). Hs-CRP: high sensitivity C-reactive protein; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index

## 5.4 Discussion

To the best of our knowledge, this is the first study to report cross-sectional and longitudinal associations between serum hs-CRP and total knee BMLs and total WOMAC knee pain in patients with knee OA. Our data demonstrated that serum hs-CRP was associated with the presence of total knee BMLs cross-sectionally, and predicted worsening knee BMLs over 2 years in patients with knee OA. Furthermore, there were significant associations between hs-CRP and total WOMAC knee pain and their changes, and the longitudinal association was partly explained by total knee BMLs. These results add to the growing evidence that the clinical course of OA is associated with systemic inflammation [26, 142, 224].

Several serum biomarkers have been correlated with OA incidence and progression and are thus proposed as diagnostic and prognostic markers for knee OA [228, 229]. Cartilage oligomeric matrix protein (COMP) is an important degradation product of articular cartilage [74] and baseline serum COMP was associated with incident radiographic knee OA over 6 years [230]. Serum hyaluronic acid (HA) concentration was positively associated with progression of Kellgren Lawrence grade in knees with and without OA [229]. Serum levels of kerantan sulphate (KS) is also a potential marker of cartilage degradation [231]. As one of the most widely- used clinical marker of systemic inflammation, serum hs-CRP has been investigated by some studies regarding its associations with incidence [179], progression [184], symptoms [232] and structures [233] of knee OA. Spector et al reported that serum CRP levels were significantly increased in women with radiographic knee OA compared with those without, and higher CRP values predicted radiographic OA progression over four years [234]. Pelletier et al. [235] found that baseline levels of CRP predicted cartilage volume loss in the medial compartment, and correlated with worsening of WOMAC pain in patients with knee pain. Further, Pearle et al observed a positive correlation between plasma CRP and histologic evidence of synovitis (inflammatory cell infiltration in synovial membrane) at the time of joint replacement in patients with OA [236]. Our recent meta-analysis revealed that serum hs-CRP levels in OA were higher than controls, although differences were modest; greater hs-CRP was associated with more knee pain but not with radiographic OA [224]. However, current evidence regarding the association and OA is mainly derived from cross-sectional studies (account for about 75% of total studies regarding to CRP and OA) [224], so whether the association is causal is not known. A previous study [181] reported that serum CRP measured 3 years prior was predictive of radiographic OA progression over 5 years, but suggested that serum CRP concentration was not good predictor of individual patient progression because the sensitivity and specificity as a marker for individuals were poor. Another study reported that baseline levels of hs-CRP did not predict incident radiographic knee OA [230]. Besides, there are few studies reporting associations between serum hs-CRP and knee BMLs (an imaging marker of knee OA) both cross-sectionally and longitudinally in OA patients.

One previous study [185] evaluated cross-sectional correlations between hs-CRP and bone marrow oedema (the former name of BMLs); it found no differences in hs-CRP between patients with and without bone marrow oedema in a symptomatic OA population. However, this study is limited by its cross-sectional design and relatively small sample size (n=30). Our current study examined cross-sectional and longitudinal associations between serum hs-CRP and BMLs in patients with knee OA, and reported positive associations between hs-CRP and total knee BMLs, independent of age, sex, BMI, intervention and co-morbidities. The associations were consistent: patients with higher baseline hs-CRP had greater prevalence of knee BMLs and greater proportion of having an increase in BMLs; patients with greater increase in hs-CRP had also greater increase in knee BMLs. These findings suggest that low grade inflammatory process may play a pivotal role in the etiology of knee BMLs and serum levels of hs-CRP may be a biomarker of progressive BMLs in knee OA.

In the current study, baseline hs-CRP was not associated with change in total knee pain over 2 years. This is inconsistent with our previous findings [225], in which serum hs-CRP was associated with worsening knee pain over 5 years in older adults. The reasons for this inconsistency are unclear, but it may be due to differences in study population, sample size and duration of follow-up. In our previous study, a total of 149 subjects with 45% prevalence of knee pain were selected and analysed at baseline and 5 years later [225] whereas in our present study, 192 OA patients with mild to moderate knee pain were included. In addition, the follow up period was 2 years rather than 5 years in the present study, which may not be long enough to observe any associations between baseline hs-CRP and increases in knee pain. Although the current study does not provide evidence of a causal relationship, hs-CRP and change in hs-CRP were consistently associated with knee pain and change in knee pain, respectively. This suggests potential involvement of inflammation in the symptoms of knee OA.

BMLs have been linked to knee pain in older subjects [23, 74, 195] and patients with knee OA [76], and so may underlie the inflammatory involvement of knee pain. In this study, we found that associations between change in hs-CRP and change in total WOMAC knee pain decreased in magnitude and became of borderline significance

after adjustment for BMLs. This suggests that the correlations between hs-CRP and total knee pain is at least partly explained by BMLs, which is consistent with our previous findings [225]. Serum levels of hs-CRP may reflect the inflammatory process in BMLs among OA patients which can be an underlying mechanisms of OA knee pain. From a clinical perspective, our results may facilitate the development of new therapeutic strategies targeting low-grade inflammation for knee OA.

Obesity can influence serum hs-CRP levels, and BMI is a major determinant of CRP [237]. The influence of obesity in the development of OA was revealed to go beyond mechanical loading, and obesity-related inflammatory cytokines (so-called adipocytokines) are implicated in the pathogenesis of OA [238]. In the current study, we also observed significant differences in BMI between subjects with higher and lower baseline hs-CRP. However, the statistically significant associations between hs-CRP and total knee BMLs as well as knee pain were not changed after adjustment for BMI both cross-sectionally and longitudinally. This is consistent with most [224] but not all previous [184] studies. Our finding suggests that low-grade inflammatory process may influence BMLs and symptoms in knee OA, independent of obesity.

A strength of the present study was the longitudinal design, which allowed assessment of a temporal relationship between serum hs-CRP and BMLs. There were several potential limitations. First, the original study was an RCT study and most participants were vitamin D deficient OA patients. Low serum levels of vitamin D may affect OA and BMLs progression [239]. However, all longitudinal associations remained significant after adjustment for intervention (vitamin D supplementation) or change in 25-OHD levels (data not shown), suggesting serum levels of vitamin D may not affect our results. Second, inclusion and exclusion criteria were applied in the RCT. Therefore, the findings may not be generalisable to all patients with knee OA. Third, other potential confounding variables such as physical activity and medication uses were not recorded in this study. Fourth, the accumulation of BML data at baseline and after 2 years may introduce bias due to BML fluctuation over short term [73]; however, associations between hs-CRP and BMLs were consistent cross-sectionally and longitudinally suggesting this is unlikely. Last, the period of 2 years follow-up may not

be sufficient to detect associations between hs-CRP and change in pain; previous studies suggest that 5 years may be required to detect the associations [225, 240].

In conclusion, serum hs-CRP is associated with knee BML scores and, to a lesser extent, pain both cross-sectionally and longitudinally in patients with knee OA, suggesting that inflammation is linked with BMLs and their associated pain.

# Chapter 6

# Associations between serum inflammatory cytokines and knee bone marrow lesions in patients with knee osteoarthritis

This manuscript has been published (Zhu *et al* Osteoarthritis & Cartilage. 2016; Nov 9 Epub ahead of print). The typeset version of the manuscript as it appeared in the journal is in Appendix 3. The text of this chapter is the same as the published version, except where changes have been requested by the examiners. Thus, there is some repetition of the methods.

## 6.1 Introduction

Osteoarthritis (OA) is the most frequent type of arthritis worldwide. It is characterized by progressive loss of cartilage, deterioration of subchondral bone and mild synovial inflammation [223]. While OA has traditionally been regarded as a non-inflammatory type of arthritis, there is a growing evidence that the clinical course of OA may be driven by systemic and localized inflammation [26, 142, 224], albeit at much lower levels than the recognised inflammatory arthropathies [10, 143].

IL-6 is an inflammatory cytokine with pro- and anti-inflammatory effects, both inside and outside of the joints [150, 151, 241]. Although emerging evidence suggests that low-level systemic inflammation is involved in OA pathogenesis [26, 152], the role of IL-6 in OA remains controversial. In a spontaneous aging model of OA, mice deficient in IL-6 displayed increased levels of cartilage loss, suggesting a potentially protective role of IL-6 in the development of OA. In contrast, higher levels of serum IL-6 were associated with higher prevalence of osteophytes in older adults [142]. Circulating levels of IL-6 have been associated with the prevalence of knee OA [143].

IL-17A and IL-17F are the prototypical members of the IL-17 cytokine family, which are produced by CD4 (+) T-helper 17 cells (Th-17) [163]. IL-17A and IL-17F share the most homology at the amino acid level [164], have overlapping but also distinct effector functions in a range of autoimmune diseases [165]. The development of CD4 (+) Th-17 cells is differentiated by IL-6, and stabilised by IL-23 [163]. Produced by antigen presenting cells (APCs), IL-23 is a heterodimeric protein composed of a p19 and a p40 subunit. Several groups have demonstrated that IL-1β, IL-6 and IL-23 promote human Th-17 cells differentiation from CD4+ cells, resulting in the expression of IL-17A, IL-17F, and IL-6 [166, 167]. Thus, IL-6, IL-23 and IL-17 form a new axis through Th-17 cells, which has an important role in autoimmunity and chronic inflammation [242-245].

Bone marrow lesions (BMLs), observed as ill-defined hyperintense signals in subchondral bone of the knees on magnetic resonance imaging (MRI) scans, are an important feature of knee OA. They are strongly associated with knee pain [75, 76],

and OA incidence [77] and progression [78, 79]. Additionally, BMLs predict knee joint space loss on x-ray [79], cartilage defect progression [55] and cartilage loss on MRI [80], as well as knee replacement surgery [81, 246]. However, thus far, the etiology of BML formation is largely unknown. An experimental study reported BMLs might originally correspond to an acute inflammatory response, oedema, contusion and/or necrosis, which were eventually replaced by permanent bone marrow remodelling such as fibrosis and myxomatous connective tissue [247]. BMLs could be caused by an inflammatory reaction to cartilage breakdown products, or other factors in intruded synovial fluid [87]. Our previous study reported that high serum hs-CRP was associated with increased knee BMLs in patients with knee OA, suggesting that systemic inflammation may play a role in the pathogenesis of BMLs in knee OA patients [248].

Although IL-6/IL-23/IL-17 axis has been implicated in the pathogenesis of many inflammatory conditions [164], its' roles in human OA are unclear. Therefore, the aim of this study was to describe cross-sectional and longitudinal associations between serum IL-6, IL-17A, IL-17F, IL-23 and knee BMLs in patients with knee OA.

## 6.2 Subjects and methods

This study was a sub-analysis from the Vitamin D Effects on Osteoarthritis (VIDEO) Study, which was a multi-centre parallel-group, randomized, placebo-controlled and double-blind clinical trial to evaluate the effects of vitamin D supplementation in patients with symptomatic knee OA and a low 25-hydroxyvitamin D (25OHD) levels. Measures of IL-6, IL-17A, IL-17F and IL-23 were made from the 192 patients (mean 63 years, range 49-79 years, female 53%) recruited in Hobart. Inclusion and exclusion criteria were the same as for the VIDEO study [202]. Briefly, eligible subjects met the American College of Rheumatology (ACR) criteria for clinical knee OA [6], and had a pain score more than 20 mm on a 100 mm visual analogue scale (VAS). They also had an ACR function class rating of I, II and III [199], and relatively good health, with a score of 0 to 2 out of a maximum score of 4 on a global investigator assessment of disease status [227], where 0 indicates very good health and 4 indicating very poor health.

Subjects were included if their serum 25OHD levels were > 12.5 nmol/L and < 60 nmol/L and were randomly assigned to receive either a monthly capsule of 50,000 IU (1.25 mg) vitamin D3 (cholecalciferol) or identical placebo for two years. Exclusion criteria included grade 3 radiographic changes according to Altman's atlas [34], severe knee pain on standing (more than 80 mm on a l00 mm VAS), contraindication to MRI, rheumatoid or psoriatic arthritis, lupus, cancer, and severe cardiac or renal impairment.

The VIDEO study was approved by the Tasmania Health and Human Medical Research Ethics Committee (reference number H1040). Written informed consent was obtained from all participants.

### 6.2.1 Inflammatory markers measurements

Serum levels of IL-6, IL-17A, IL-17F and IL-23 were measured at baseline and after 24 months using enzyme-linked immunosorbent assay [10]. The limits of detection are 0.49 pg/ml, 1.84 pg/ml, 14.6 pg/ml and 34.4 pg/ml respectively. The proportion of participants with IL-6 below limit of detection was 17%. The majority of study participants had IL-17A, IL-17F and IL-23 levels below limits of detection, with the proportions of measurements below their limits of detection for IL-17A, IL-17F and IL-23 were 78%, 60% and 77%, respectively. The coefficients of variation (CVs) were 5.8%-6.3%.

### 6.2.2 Assessment of bone marrow lesions

BMLs in the diseased knee, or of the less painful knee if both knees (aiming to avoid "ceiling effects" of the treatment on disease outcomes when designed in original randomized controlled trail (RCT) study) were affected were assessed on MR images. These were acquired with a 1.5T whole-body magnetic resonance unit (Picker, Cleveland, Ohio, USA) using a commercial transmit-receive extremity coil at baseline and 24 months later. Image sequence included the following: Fat-saturated T2-weighted fast spin echo (FSE), flip angle 90º, repetition time 3067 ms, echo time 112 ms, field of view 16 cm, 45 partitions, 228×256-pixel matrix; sagittal images were obtained at a partition thickness of 2 mm.

BMLs were measured by one trained observer (ZZ) using the modified whole organ MRI score (WORMS) [125]. In the modified WORMS, the medial and lateral tibial and femoral compartments are divided into three sub-regions (anterior, central, posterior), and the tibia has an additional subspinous sub-region which represents the portion of the tibia beneath the tibial spines. Thus, a total of 15 sub-regions were scored for each knee [125].

BMLs were scored according to the maximal percentage of bone area that the lesion occupied within the total sub-region. We scored grade 0 if no BMLs were present; grade 1 if lesion size=<25% of the subregion; grade 2, 25% to 50% of the subregion; grade 3, > 50% of the subregion [125]. Total knee BML scores were obtained by summing the BML scores of all the sites, giving a potential total knee BMLs score range of 0 to 45. Baseline and follow-up MRIs were scored in pairs in chronological order to minimise measurement error [74]. The intra-rater reliability (ICC=0.93-0.98) and inter-rater reliability (ICC=0.91-0.97) were excellent. Presence of BMLs in the whole knee was defined as a BML score of ≥1 at any subregion. For longitudinal analyses we defined an increase in BML score of greater than one between baseline and follow-up to be the outcome of interest.

### 6.2.3 Anthropometrics

Height was measured to the nearest 0.1 cm (with shoes, socks, and headgear removed) using a stadiometer. Weight was measured to the nearest 0.1 kg (with shoes, socks, and bulky clothing removed) using a single pair of electronic scales (Model 707; Seca Delta, Hamburg, Germany) that were calibrated using a known weight at the beginning of each clinic. Body mass index (BMI) [weight (kg)/height$^2$ ($m^2$)] was calculated.

### 6.2.4 25OHD assays

Serum 25OHD was assayed by the Liaison method at baseline, utilizing a direct competitive chemiluminescent immunoassays (DiaSorin Inc, Stillwater, Minnesota, USA). The intra-assay and inter-assay coefficients of variation were 3.2% and 6.0%, respectively [202].

### 6.2.5 Data analysis

Student's t-tests, $\chi^2$ tests or Wilcoxon rank-sum test (when appropriate) were used to compare means, proportions or median between those with or without baseline knee BMLs.

Baseline IL-6 was highly right-skewed, hence quarters were used for subsequent analyses, the other cytokines were dichotomised at the highest quarters. Baseline BML score had a right-skewed and zero-inflated distribution, thus multivariable negative binomial regression models were used to determine associations between cytokines and total knee BML scores, adjusted for age, sex, BMI and vitamin D level. Negative binomial regressions were used to address the problems of the unusual distribution of the BML score variable at baseline, which was bounded (at zero), highly skewed, and with a large proportion of zero scores. Alternative models were investigated but ultimately rejected. Serum levels of 25-(OH)D were associated with inflammatory markers including IL-6, IL-17, IL-23 [249-251], as well as BMLs [252, 253]; therefore, 25-(OH)D levels were used for adjustment as p potentially confounding factor.

Log binominal regression was used to assess associations between baseline cytokines and presence of an increase in total knee BMLs, adjusted for age, sex, BMI, vitamin D levels and baseline total knee BMLs. Log-binomial regression models were used to estimated relative risks where the outcome was dichotomous. Logistic regression was also considered for this type of analysis but rejected since the resultant odds rations would overestimate the more appropriate relative risks due to the common nature of the outcome (an increase in BMLs from baseline to follow-up). Interactions between sex and cytokines were tested in all multivariable models. When significant interactions were identified, estimates are presented separately for each sex.

A *p* value less than 0.05 (two tailed) was regarded as statistically significant. All statistical analyses were performed on Stata version 12.0 for Windows (StataCorp, College Station, TX, USA).

## 6.3 Results

### 6.3.1 Characteristics of the study population at baseline.

A total of 192 subjects (53% female) aged between 49 and 79 years old (mean 63 years) were included. 80% had BMLs visible on MR imaging at baseline. Characteristics of the participants at baseline are presented in Table 1. No statistically significant differences were found between participants with and without baseline BMLs in terms of age, sex, BMI and baseline serum cytokine levels (Table 1).

**Table 6. 1** Characteristics of participants at baseline, by presence or absence of BMLs at baseline

| | Without Baseline BMLs (N=36) | With Baseline BMLs (N=156) | p value |
|---|---|---|---|
| Age (years) | 62.2 ± 7.3 | 63.3 ± 7.0 | 0.32 |
| Females (%) | 54% | 49% | 0.42 |
| BMI (kg/m$^2$) | 30.1 ± 6.0 | 29.5 ± 4.8 | 0.38 |
| 25-(OH) D (nmol/L) | 44.4 ± 13.1 | 45.6 ± 13.6 | 0.47 |
| IL-6 (pg/ml) | 1.33 (0.4, 3.7) | 1.16 (0.4, 2.5) | 0.65 |
| IL-17A (%) * | 20% | 25% | 0.48 |
| IL-17F (%) * | 36% | 39% | 0.97 |
| IL-23 (%) * | 17% | 28% | 0.08 |

Two-tailed Student's t tests were used for differences between means, $\chi^2$ tests were used for proportions (percentages) and Wilcoxon rank-sum tests were used for differences between medians. Results are mean ± SD or median (IQR) except for percentage. BMI, body mass index. 25-(OH)D: 25-hydroxyvitamin D; IL: interleukin. * Proportions of measurements above their limits of detection. Limits of detection: IL-6, 0.49 (pg/ml); IL-17A, 1.84 (pg/ml); IL-17F, 14.6 (pg/ml); IL-23, 34.4 (pg/ml).

### 6.3.2 Associations between IL-6 and BMLs.

Patients in the higher quarters of baseline IL-6 had higher baseline total knee BML scores (Figure 1a), and were more likely to have an increase in total knee BMLs over 2 years (Figure 1b).

Table 2 describes cross-sectional and longitudinal associations between serum IL-6 and total knee BMLs over 2 years. Baseline IL-6 (quarters) were statistically significantly associated with baseline total knee BML score, after adjustment for age, sex, BMI and vitamin D levels (Table 2). Higher baseline IL-6 (quarters) were statistically significantly associated with greater risks of increased BML scores over 2 years, after adjustment for age, sex, BMI, vitamin D levels and baseline total knee BMLs, in log binomial regression analyses (Table 2). There were no statistically significant interactions between sex and IL-6 (data not shown), therefore men and women were combined for analyses.

**Table 6. 2** Cross-sectional and longitudinal associations between serum IL-6 and total knee BMLs/an increase in BMLs over 2 years

| | | **Univariable** | | **Multivariable** |
|---|---|---|---|---|
| ***Total knee BML scores*** | RM† | 95% CI | RM† | 95% CI |
| Baseline IL-6 (quarter)=1 | | Ref | | Ref |
| Baseline IL-6 (quarter)=2 | 1.23 | (0.83, 1.83) | 1.24 | (0.83, 1.86) |
| Baseline IL-6 (quarter)=3 | **1.63** | **(1.11, 2.40)** | **1.68** | **(1.12, 2.52)** |
| Baseline IL-6 (quarter)=4 | **1.61** | **(1.08, 2.39)** | **1.62** | **(1.09, 2.42)** |
| p for the trend | | **p<0.01** | | **p*<0.01** |
| | | | | |
| ***An increase in BMLs*** | RR | 95% CI | RR | 95% CI |
| Baseline IL-6 (quarter)=1 | | Ref | | Ref |
| Baseline IL-6 (quarter)=2 | 1.21 | (0.60, 2.43) | 1.25 | (0.62, 2.50) |
| Baseline IL-6 (quarter)=3 | 1.36 | (0.70, 2.67) | 1.58 | (0.80, 3.12) |
| Baseline IL-6 (quarter)=4 | 1.74 | (0.92, 3.29) | **1.90** | **(1.01, 3.58)** |
| p for the trend | | p=0.07 | | **p**=0.05** |

The dependent variable: baseline total knee BML score in negative binominal models, or an increase in total knee BMLs in log binominal models. The independent variable: quarters of IL-6. †Ratio of means presented from negative binomial model; *Adjustment for age, sex, BMI and vitamin D levels; ** Adjusted for age, sex, BMI, vitamin D levels and baseline total knee BMLs. Bold denoted statistical significance ($p<0.05$).

### 6.3.3 Associations between IL-17A, IL-17F, IL-23 and BMLs.

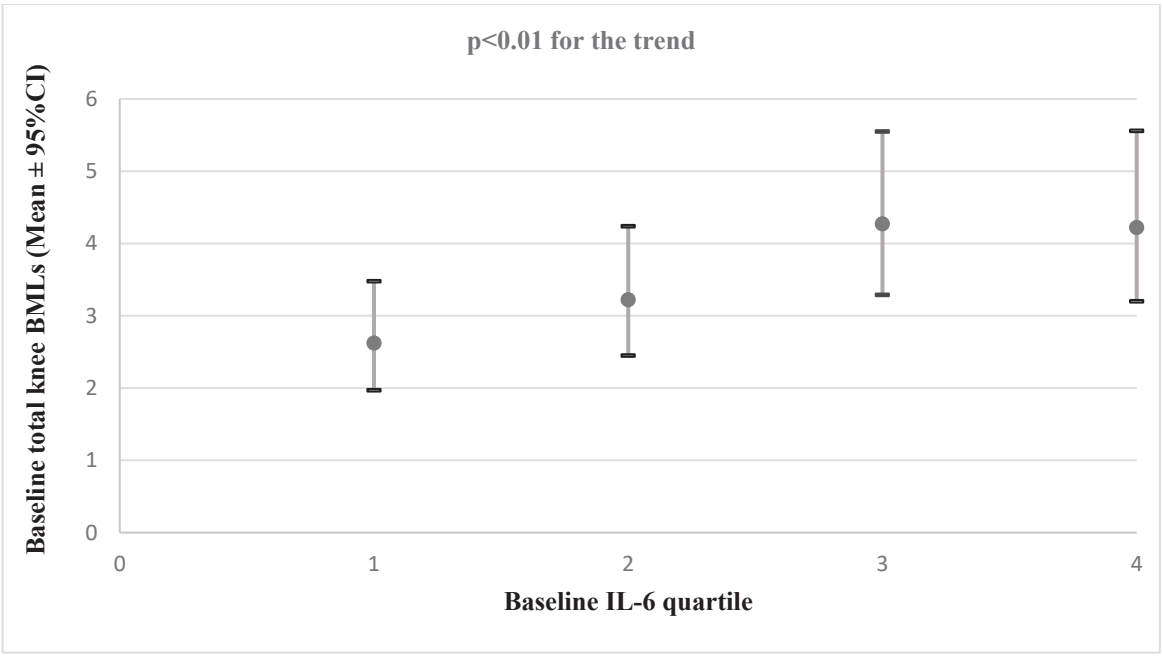
Table 3 describes cross-sectional and longitudinal associations between serum IL-17A, IL-17F and IL-23 and total knee BMLs over 2 years in males and females. Interactions between sex and IL-17F, IL-23 on BMLs were statistically significant (both $p<0.1$), so men and women were analysed separately. Additionally, no statistically significant sex interaction was found for IL-17A, however since this interleukin is part of the same family as the others and they are located adjacent to each other and exhibit a similar cysteine knot configuration, we present results separately for males and females also.

Dichotomized baseline IL-17A, IL-17F and IL-23 (highest quartile vs others or detectable vs undetectable) were not associated with baseline total knee BMLs in females or males, before or after adjustment for age, BMI and vitamin D levels, in multivariable negative binominal regression analyses (Table 3). In females, those with higher baseline IL-17F and IL-23 had 1.9-fold and 2.1-fold higher risks of increased BML scores, after adjustment for age, BMI, vitamin D levels and baseline total knee BMLs, in log binomial regression analyses (Table 3, Figure 2). However, no statistically significant associations were found in male group (Table 3). IL-17A was not statistically significantly associated with an increase in BMLs in either females or males (Table 3).

**Table 6. 3** Cross-sectional and longitudinal associations between Th17 cytokines and total knee BMLs/an increase in BMLs over 2 years, by sex

| | **Multivariable analysis in Males** | | **Multivariable analysis in Females** | |
|---|---|---|---|---|
| ***Total knee BML scores*** | RM† | 95% CI* | RM† | 95% CI* |
| Baseline IL-17A | 1.28 | (0.68, 1.89) | 0.95 | (0.57, 1.57) |
| Baseline IL-17F | 1.31 | (0.72, 1.89) | 1.05 | (0.65, 1.68) |
| Baseline IL-23 | 1.34 | (0.74, 1.94) | 0.92 | (0.55, 1.54) |
| | | | | |
| ***An increase in BMLs*** | RR | 95% CI** | RR | 95% CI** |
| Baseline IL-17A | 0.81 | (0.13, 1.50) | 1.53 | (0.79, 2.94) |
| Baseline IL-17F | 0.83 | (0.18, 1.48) | **1.85** | **(1.04, 3.28)** |
| Baseline IL-23 | 0.88 | (0.19, 1.57) | **2.10** | **(1.16, 3.79)** |

The dependent variable: baseline total knee BML score in negative binominal models, or an increase in total knee BMLs in log binominal models. The independent variables: baseline IL-17A, IL-23 (dichotomised at cut-off points of limits of detection), and IL-17F (highest quarter vs others). †Ratio of means presented from negative binomial model; *Adjusted for age, BMI and vitamin D levels; ** Adjusted for age, BMI, vitamin D levels and baseline total knee BMLs; Bold denoted statistical significance ($p<0.05$).




**(a)**

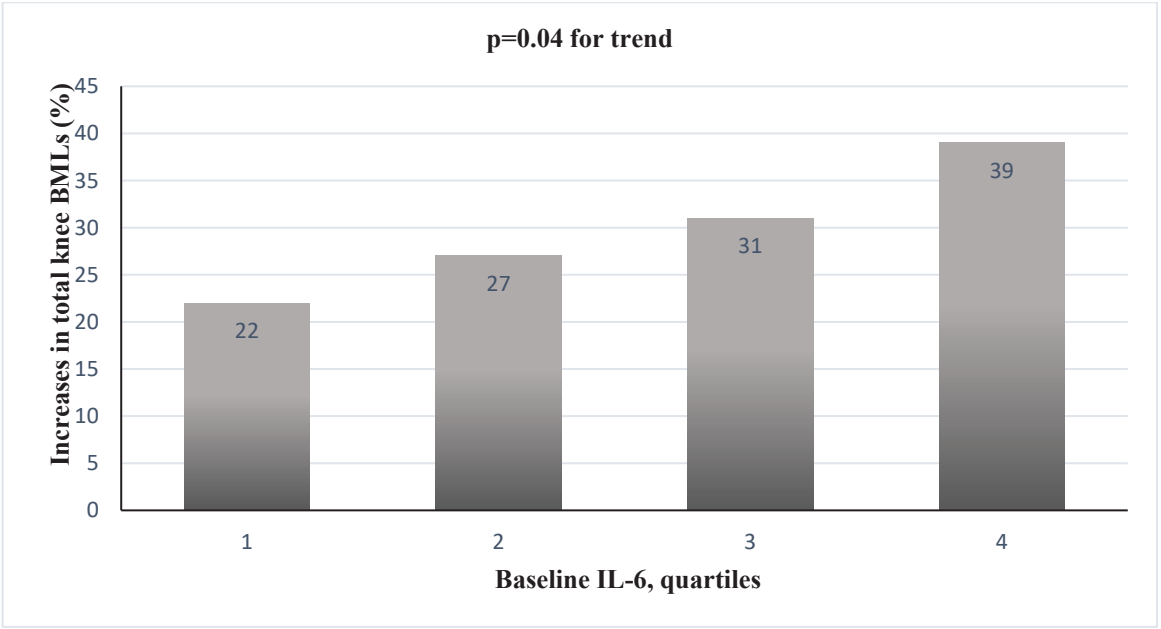



**Figure 6. 1** Association between IL-6 and knee BMLs in total sample

(a) Baseline total knee BMLs (mean +/- 95%CI), by quarters of baseline IL-6; (b) An increase in total knee BMLs over 2 years by quarters of baseline IL-6. BMLs: bone marrow lesion.

**(b)**

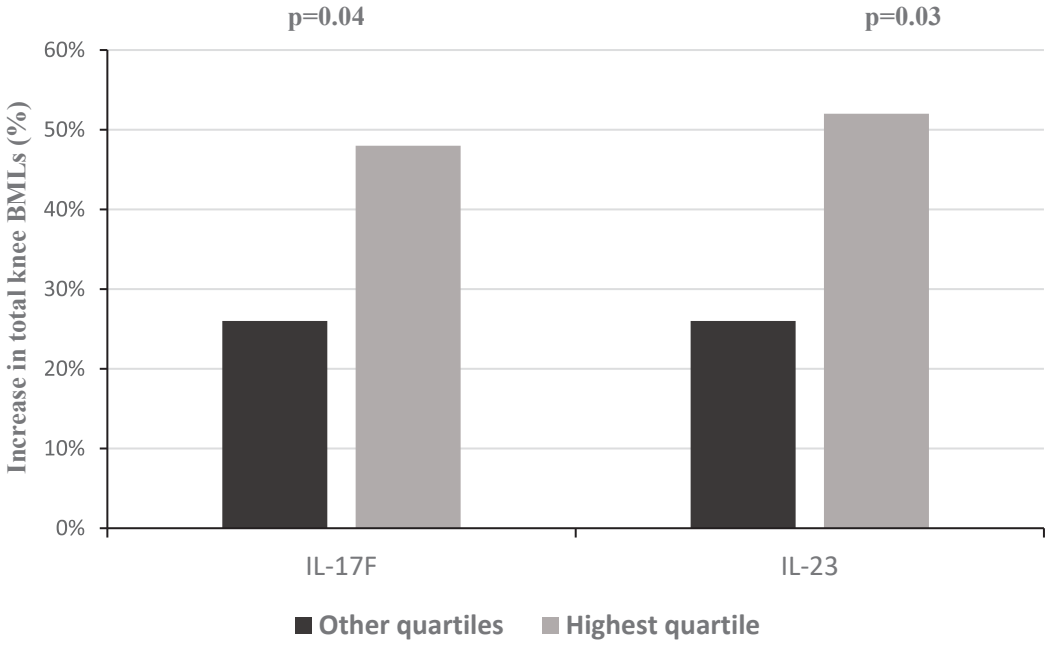



**Figure 6. 2** Association between IL-17F, IL-23 and an increase in total knee BMLs over 2 years in females

## 6.4 Discussion

This study is the first, to our knowledge, to assess longitudinal associations between IL-6, IL-23 and IL-17, and knee BMLs in patients with knee OA. Our data showed that higher serum IL-6 was associated with greater likelihood of an increase in knee BMLs over 2 years, and that high levels of IL-17F and IL-23 predicted an increase in knee BMLs in females (but not males) with knee OA. These results add to the growing evidence that systematic inflammation is involved in the pathogenesis of knee OA, particularly among females [26, 142].

IL-6 levels are elevated locally at sites of inflammation, and can activate B cells, T cells and other inflammatory cells [254]. Increased IL-6 expression has also been observed in subchondral bone and osteophytes of subjects with knee OA [255]. In our present study, associations between serum IL-6 and total knee BMLs as well as increases in knee BMLs over 2 years were independent of potential confounders including age, sex,

BMI and vitamin D levels, suggesting that serum IL-6 is involved in the pathogenesis of BMLs in knee OA patients and predicts BML development/progression. This is in line with our previous report in which a higher circulating levels of IL-6 was an independent predictor of worsening knee pain [225].

A number of investigations have demonstrated roles for Th17 cytokines in the aetiology and progression of rheumatoid arthritis (RA) [256]; the cytokines are expressed by rheumatoid synovium and can induce inflammation and osteoclastic bone resorption [257]. IL-17A and IL-17F are pro-inflammatory cytokines, and have regulatory roles in host defence and chronic inflammation, resulting in tissue damage and autoimmunity [258, 259]. However, little is known about the role of IL-17 in OA. Chen et al [171] reported a positive association between knee OA severity and IL-17 concentration in synovial fluid, but not in serum. Liu et al [172] showed that synovial IL-17 level was correlated with severity of knee pain, but not with radiographic severity. These studies were limited by their cross-sectional design, small sample size and lack of adjustment for potential confounders. In our present study, we found that baseline IL-17F predicted increased BML scores in females but not males. Associations between IL-17A and BMLs were consistently positive in both females and males, but none reached statistical significance. IL-17A and IL-17F are expected to have similar physiological effects because they are located adjacent and exhibit a similar cysteine knot configuration [260]. However, our findings suggest that IL-17F may play a more important role in human knee OA aetiology than IL-17A, but the underlying reasons for this difference are unknown. In this study, we also found that baseline serum IL-23 predicted increased BMLs in females but not males; this association was independent of potential confounders such as age, BMI and vitamin D levels. Combined with the associations that we found between IL-6, IL-17F and knee BMLs in this study, these results suggest that IL-6/IL-23/IL-17 axis may play a role in the aetiology and progression of knee OA, at least in females. Further studies investigating other OA- related features (meniscal tears, ligamentous lesions, cartilage defects, etc.) and IL-6/IL-23/IL-17 axis are required to provide more comprehensive understanding of the relationship between inflammation and knee OA progression.

The reasons for why we observed a sex difference for the association between IL-17F/IL-23 and BMLs, but not between IL-6 and BMLs are unclear. It may reflect the influence of sex hormones on the relationships of IL-17/IL-23 and knee BMLs. We previously reported that serum levels of IL-6 were associated with hip radiographic changes in females but not in males [145], which was inconsistent with the present study. This may imply that there are different inflammatory pathways between hip OA and knee OA in the relationship with IL-6.

This study has several limitations. First, the original study was a randomized controlled trail (RCT); therefore, results could be affected by the intervention (vitamin D supplementation). However, all associations from longitudinal analyses remained significant after adjustment for vitamin D levels. Secondly, giving that the majority of subjects had cytokine levels under the limits of detection for most cytokines, we dichotomized predictors rather than using them as continuous variables. Therefore, we cannot completely exclude the possibility that the relationships are model-specific, and replications using predictors with higher sensitivity are required in future studies. Third, we did not measure ligamentous and meniscal status that may be risk factors of BMLs [261]; however, there is no evidence showing that serum levels of inflammatory cytokines are associated with meniscal and/or ligamentous status, so meniscal and/or ligamentous status seem unlikely to be potential confounders for the associations between inflammatory cytokines and BMLs. Lastly, as inclusion and exclusion criteria were applied in the original RCT design, the generalizability to the general knee OA population needs to be confirmed.

In conclusion, while serum IL-6 is associated with increased knee BMLs in both female and male OA patients, serum IL-17F and IL-23 only predict increased knee BML scores in females. These suggest that inflammation is involved in BML pathogenesis in knee OA, with the IL-6/IL-23/IL-17 axis having a role particularly in women.

# Chapter 7:

# Association between MRI-detected osteophytes and changes in knee structures and pain in older adults: a cohort study

This manuscript has been published (Zhu *et al* Osteoarthritis Cartilage. 2017; Jan 21 S1063-4584 (17)). The typeset version of the manuscript as it appeared in the journal is in Appendix 4. The text of this chapter is the same as the published version, except where changes have been requested by the examiners. Thus, there is some repetition of the methods.

## 7.1 Introduction

Osteoarthritis (OA) is the most common type of arthritis, with prevalence estimates expected to increase dramatically worldwide due to aging and increasingly obese populations [2, 16]. There is a pressing need for biomarkers that can identify or predict the potential structural abnormalities and subsequent symptoms of disease, which would aid decision-making at both individual and community levels. Osteophyte (OP) formation is one of the common features of OA [6, 262, 263]. However, there are only modest correlations between knee OPs and clinical features [47, 103]. Additionally, change in knee symptoms is poorly predicted by OPs on baseline radiographs [264].

Magnetic resonance imaging (MRI) is a non-invasive multiplanar tomographic tool that has been introduced to evaluate knee osteoarthritic changes such as bone marrow lesions (BMLs) [265], cartilage defects [190] and cartilage volume [266]. Although MRI can assess OPs in locations that are not easily visualised by conventional radiography [105, 188], and at greater sensitivity than radiographs for detection of early formation of OP [104], few data are available to compare the prevalence of OPs detected by MRI and radiography in population-based samples. It has been shown that greater size of MRI-detected OPs correlated with higher Kellgren-Lawrence score [63] and increased knee pain [110], and cross-sectional studies have suggested that increasing size and presence of MRI-defined OPs was associated with severity of knee OA [9, 63, 111] as well as presence of pain. However, longitudinal studies are rare [110, 267]. Thus, the purposes of current study are to describe cross-sectional and longitudinal associations between MRI-detected OPs and knee structural abnormalities over 2.6 years as well as knee pain during 5 years in older adults.

## 7.2 Material and methods

### 7.2.1 Subjects

This study used data from the Tasmania Older Adult Cohort (TASOAC) study, which is an ongoing, prospective, population-based study that aimed to identify the

environmental, genetic, and biochemical factors associated with the development and progression of OA. Participants between 50 and 80 years old were randomly selected from the electoral roll in Southern Tasmania (population 229, 000) using sex-stratified random sampling (response rate 57%). Participants were excluded if they were institutionalised or had contraindications to MRI. The Southern Tasmania Health and Medical Human Research Ethics Committee approved the study, and written informed consent was obtained from all participants. Baseline examinations were taken between February 2002 and September 2004, and follow-up measures were taken at approximately 2.6 and 5.1 years later.

### 7.2.2 Anthropometrics

Height was measured to the nearest 0.1 cm (with shoes, and headgear removed) using a stadiometer. Weight was measured to the nearest 0.1 kg (with shoes, socks, and bulky clothing removed) by using a single pair of electronic scales (Delta Model 707, Seca, Hamburg, Germany) that were calibrated using a known weight at the beginning of each clinic. Body mass index (BMI, weight (kg)/height$^2$ (m$^2$)) was also calculated.

### 7.2.3 WOMAC pain assessment

Knee pain was assessed using the Western Ontario McMaster Osteoarthritis Index (WOMAC) [204] at baseline, 2.6 and 5 years later using a 10-point scale from 0 (no pain) to 9 (most severe pain). The 5 subscales (walking on flat surface, going up/down stairs, at night, sitting/lying and standing upright) were assessed separately and summed to create a total pain score (0 to 45). The presence of knee pain was defined as total WOMAC pain score of 1 or greater [268]. Change in knee pain score was calculated as follow-up value - baseline value. An increase in total WOMAC pain was defined as a change in WOMAC pain score of ≥1.

### 7.2.4 X-ray assessment

A standing anteroposterior semiflexed view of the right knee with 15° of fixed knee flexion was performed in all subject at baseline [269]. Joint space narrowing (JSN) and

radiographic osteophytes (OPs) were scored at the medial tibia, medial femur, lateral tibia and lateral femur on a scale of 0-3 (0=normal, 3= severe) according to the Osteoarthritis Research Society International (OARSI) atlas developed by Altman et al. [31] OP score in the whole knee was the highest score of all compartments of the knee. The presence of radiographically- detected OP was defined as the OP score ≥ 1 on X-ray. The presence of radiographic OA (ROA) was defined as any JSN or OP score of ≥1. Each score was determined by two readers (VS & HC) who simultaneously assessed the radiograph with immediate reference to the atlas. Intraobserver repeatability was tested in 40 subjects one month apart with intraclass correlation coefficients (ICCs) of 0.65-0.85 [23].

### 7.2.5 Magnetic Resonance Imaging

MRI scans of the right knees were performed on two occasions (baseline and 2.6 years later) and imaged in the sagittal plane on a 1.5-T whole body magnetic resonance unit (Picker, Cleveland, OH) using a commercial transmit-receive extremity coil. The image sequences were used as follows: (1) a T1-weighted fat saturation 3D gradient recall acquisition in the steady state; flip angle 30°; repetition time 31 ms; echo time 6.71 ms; field of view 16 cm; 60 partitions; 512×512 matrix; acquisition time 11 min 56 s; one acquisition. Sagittal images were obtained at a partition thickness of 1.5 mm and an in-plane resolution of 0.31×0.31 (512×512 pixels). (2) a T2-weighted fat saturation 3-D fast spin echo, flip angle 90, repetition time 3067 ms, echo time 112 ms, field of view 16 cm, 15 partitions, 228x256-pixel matrix; sagittal images were obtained at a partition thickness of 4 mm with a between-slices gap of 0.5 to 1.0 mm. The image database was transferred to an independent computer workstation using the software program Osirix (University of Geneva, Geneva, Switzerland) as previously described [116, 191].

### 7.2.6 MRI-detected OP assessment

MRI-detected OPs were measured (Supplementary Figure 1) by ZZ according to the Knee Osteoarthritis Scoring System [196] where OPs are defined as focal bony excrescences, seen on sagittal, axial or coronal images, extending from a cortical

surface. OPs were measured using the following scale: grade 0, absent; grade 1, minimal (<3 mm); grade 2, moderate (3-5 mm); grade 3, severe (>5 mm). Size was measured from the base (distinguished from that of adjacent articular cartilage with a normal MRI appearance) to the tip of the OP [188] at each of the following 14 sites: the anterior (a), central weight- bearing (c) and posterior (p) margins of the femoral condyles and tibial plateaus, and the medial (M) and lateral (L) margins of the patella [125]. The worst score of each individual site in the relevant compartment (or whole knee) was regarded as the OP score in that compartment (or whole knee). MRI-detected OP was considered as present if OP score of ≥1. Intra-observer reliability (expressed as ICC) was 0.94-0.97 and inter-observer reliability was 0.90-0.96.

### 7.2.7 Cartilage defects

Cartilage defects were assessed on T1-weighted MRI and graded at medial tibial, lateral tibial, medial femoral, lateral femoral and patellar regions as previously described [126, 129]. as follows: grade 0, normal cartilage; grade 1, focal blistering and low-signal intensity change with an intact surface and bottom; grade 2, irregularities on the surface or bottom and loss of thickness of less than 50%; grade 3, deep ulceration with loss of thickness of more than 50%; grade 4, full thickness cartilage loss with exposure of subchondral bone [126]. The worst score of each individual site in the relevant compartment (or whole knee) was regarded as the cartilage defect score in that compartment (or whole knee). The presence of cartilage defects was defined as a cartilage defect score of ≥2 at any site. An increase in cartilage defects was defined as a change in cartilage defects of ≥1. Intra-observer reliability (expressed as ICC) was 0.89-0.94 and inter-observer reliability was 0.85-0.93 [126].

### 7.2.8 Cartilage volume

Knee cartilage volume was measured on T1-weighted images by a single trained observer as previously described [193, 194]. The volumes of individual cartilage plates (medial tibial, lateral tibial) were isolated from the total volume by manually drawing disarticulation contours around the cartilage boundaries on a section by section basis.

These data were resampled by means of bilinear and cubic interpolation (area of 312×312) um and 1.5 mm thickness, continuous sections) for the final 3-dimensional rendering. Changes in cartilage volume were calculated as: percentage change per annum= [(follow-up volume – baseline volume)/baseline cartilage volume]/time between 2 scans in years × 100. The coefficient of variation (CV) for cartilage volume measures was 2.1% to 2.6% [193, 194].

### 7.2.9 Bone marrow lesions

Subchondral BMLs were defined as discrete areas of increased signal adjacent to the subcortical bone on T2-weighted MRI and scored at medial tibial, lateral tibial, medial femoral, lateral femoral, medial patellar and lateral patellar regions using a modified version of Whole-Organ Magnetic Resonance Imaging Score (WORMS): grade 0, absence of BML; grade 1, area smaller than 25% of the region; grade 2, area between 25% to 50% of the region; grade 3, area larger than 50% of the region [125]. The worst score of each individual site in the relevant compartment (or whole knee) was regarded as the BML score in that compartment (or whole knee). An increase in BMLs was defined as a change in BMLs of ≥1. The inter-reader reliability of this BML scoring system has been shown to be excellent [71, 195].

### 7.2.10 Statistical analysis

Student t or $\chi^2$ tests were used to compare means or proportions between those with and without baseline MRI-detected total knee OP. Site-specific associations were defined as the associations within the same site or compartment. Multivariable linear regression analyses were used to examine the site-specific associations between baseline MRI-detected OPs (independent variables) and knee cartilage volume or change in cartilage volume (dependent variables), after adjustment for age, sex, BMI, cartilage defects and BMLs. Multivariable log binominal regression analyses were used to assess the site-specific associations between baseline MRI-detected OPs (independent variable) and presences of knee cartilage defect/BMLs as well as increases in cartilage defects/BMLs (dependent variables) over 2.6 years, before and after adjustment for age, sex, BMI,

cartilage volume (if cartilage defects or BMLs), cartilage defect (if cartilage volume or BMLs) and BMLs (if cartilage defects or cartilage volume). Sensitivity analyses were performed by repeating the analyses in those without radiographically-detected OPs. Standard diagnostic checks of model fit and residuals were made and showed that the residuals of baseline and absolute changes of WOMAC knee pain scores were not normally distributed. Therefore, multivariable log binominal regression analyses were also used to evaluate cross-sectional and longitudinal associations between baseline MRI-detected osteophytes and WOMAC knee pain over 2.6 and 5 years (yes vs no at baseline, increase vs no increase over years), both after adjustment for age, sex, BMI, cartilage defects and BMLs. All statistical analyses were performed on Stata version 12.0 for Windows (StataCorp, College Station, TX, USA).

A *p*-value < 0.05 (2-tailed) or a 95% confidence interval (CI) not including the null point (for linear regression) or 1 (for log binominal regression) was considered statistically significant.

## 7.3 Results

### 7.3.1 Distribution of X-ray and MRI-detected OPs

At baseline, 895 subjects were included for MRI assessments of OP. Mean age was 62.4 years, mean BMI was 27.7 and 50% were females. 406 subjects completed MRI measures at 2.6 years' follow-up but the rest discontinued MRI measures due to decommissioning of the MRI scanner in the local hospital. WOMAC knee pain data were available at baseline, 2.6 (n=874) and 5 years' follow-up (n=751). There were no significant differences in demographic factors, cartilage defects, BMLs, cartilage volume and radiographic OA (ROA) between participants who completed and did not complete MRI measures at baseline [210]. A total of 837 participants had readable x-ray and MRI images out of 895 baseline participants. The frequencies of OP grades detected by radiography and MRI are presented in Table 7.1/Table 7.2 85% of participants had MRI-detected OPs at baseline, while only 10% of participants had radiographically-detected OPs. 439 of 755 (58%) participants without

radiographically-detected OPs exhibited modest MRI-detected OPs (grade 1), and 189 of 755 (25%) participants without radiographically-detected OPs showed larger MRI-detected OPs (grade 2 and 3). In contrast, only 2 out of 129 participants without MRI-detected OPs showed radiographically-detected OPs. Patellar site has the most frequent MRI-detected OPs at baseline (data not shown).

**Table 7. 1** Distribution of x-ray and MRI-detected OP scores

| | | Total MRI OP scores | | | | |
|---|---|---|---|---|---|---|
| | | **0** | **1** | **2** | **3** | **Total** |
| **Total x-ray OP scores** | **0** | 127 | 439 | 132 | 57 | 755 |
| | **1** | 0 | 13 | 11 | 22 | 46 |
| | **2** | 2 | 3 | 2 | 20 | 27 |
| | **3** | 0 | 0 | 0 | 9 | 9 |
| | **Total** | 129 | 455 | 145 | 108 | 837* |

*Only 837 had readable x-ray and MRI films.

**Table 7. 2** Sensitivity and specificity of MRI detected OP

| | | X-ray detected OP | | |
|---|---|---|---|---|
| | | **No** | **Yes** | **Total** |
| **Any MRI-OP** | **No** | 127 (16.8%) | 2 (2.4%) | 129 |
| | **Yes** | 628 (83.2%) | 80 (97.6%) | 708 |
| | **Total** | 755 | 82 | 837* |

*Only 837 had readable x-ray and MRI films. X-ray detected osteophyte (OP) was used as the reference

### 7.3.2 Baseline characteristics of participants

The baseline characteristics of the participants are shown in Table 7.3. Compared with those without baseline MRI-detected OPs, those with baseline MRI-detected OPs were older, and had more proportion of males, higher weight and BMI, and larger lateral

tibial bone area. Additionally, participants with baseline MRI-detected OPs had significant less patellar cartilage volume, and higher prevalence of cartilage defects, BMLs and knee pain. The differences in prevalence of joint space narrowing and ROA between those with and without baseline MRI-detected OPs were of borderline significance (Table 7.3).

**Table 7. 3** Characteristics of participants at baseline

| | Any MRI osteophytes in total knee | | p-value |
|---|---|---|---|
| | Absent | Present | |
| | N=129 | N=708 | |
| Age (year) | **60.3±6.4** | **62.7±7.5** | **<0.01** |
| Female sex (%) | **58** | **49** | **0.05** |
| Weight (kg) | **72.4 ± 12.5** | **78.6 ± 14.8** | **<0.01** |
| BMI (kg/m²) | **26.3 ± 3.8** | **27.9 ± 4.7** | **<0.01** |
| Patella cartilage volume (ml) | **3.4±0.9** | **3.2±0.9** | **0.02** |
| Total tibial cartilage volume (ml) | 5.0 ± 1.2 | 5.1 ± 1.2 | 0.66 |
| Medial tibial bone area (cm²) | 21.8±16.4 | 21.0±3.1 | 0.23 |
| Lateral tibial bone area (cm²) | **11.8±2.0** | **12.2 ±2.2** | **0.03** |
| Any joint space narrowing (%) | 52 | 61 | 0.07 |
| Any cartilage defects (%) | **17** | **59** | **<0.01** |
| Baseline cartilage defects score, n (%) | | | |
| 1 | 105 (81) | 294 (41) | |
| 2 | 18 (14) | 215 (30) | |
| 3 | 4 (3) | 145 (21) | |
| 4 | 2 (2) | 54 (8) | |
| Any BMLs (%) | **21** | **37** | **<0.01** |
| Baseline BML score, n (%) | | | |
| 0 | 101 (78) | 446 (63) | |
| 1 | 27 (21) | 183 (26) | |
| 2 | 1 (1) | 67 (9) | |
| 3 | 0 (0) | 12 (2) | |
| Knee pain present (%) | **43** | **53** | **0.03** |
| Radiographic OA (%) | **52%** | **61%** | **0.05** |

Two-tailed t tests were used for differences between means, and $\chi 2$ tests were used for proportions (percentages). Significant differences are shown in bold. Mean ± SD except for percentages. Radiographic OA was defined using Osteoarthritis Research Society International definition with a total

score of >=1. BMI: body mass index; OA: osteoarthritis; BML: bone marrow lesions; MTF: medial tibiofemoral; LTF: lateral tibiofemoral

### 7.3.3 Associations with structural changes

Cross-sectionally, higher grades of baseline MRI-detected OPs in medial tibiofemoral, lateral tibiofemoral and patellar compartments were significantly and site-specifically associated with higher prevalences of cartilage defects, after adjustment for age, sex, BMI, baseline BMLs and cartilage volume (Table 7.4). Longitudinally, higher grades of baseline MRI-detected OPs were site-specifically associated with greater risks of any increase in cartilage defects in all compartments except for patellar site, after adjusted for covariates (Table 7.4, Figure 7.1a).

**Table 7. 4** Site-specific associations between baseline MRI-detected osteophytes and baseline/increases in knee cartilage defects

| | Multivariable* PR (95% CI) | p | Multivariable** PR (95% CI) | p |
|---|---|---|---|---|
| ***Presence of cartilage defects at baseline N=895*** | | | | |
| Medial tibiofemoral | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |
| Grade 1 | **2.70 (1.98, 3.69)** | **<0.01** | **2.61 (1.91, 3.56)** | **<0.01** |
| Grade 2 | **4.51 (3.26, 6.25)** | **<0.01** | **4.11 (2.95, 5.74)** | **<0.01** |
| Grade 3 | **7.06 (5.45, 9.13)** | **<0.01** | **6.01 (4.50, 8.02)** | **<0.01** |
| *P for trend* | | | | **<0.01** |
| Lateral tibiofemoral | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |
| Grade 1 | **2.60 (1.73, 3.90)** | **<0.01** | **2.41 (1.61, 3.60)** | **<0.01** |
| Grade 2 | **6.29 (4.11, 9.65)** | **<0.01** | **4.80 (3.09, 7.45)** | **<0.01** |
| Grade 3 | **10.5 (7.18, 15.3)** | **<0.01** | **7.46 (5.00, 11.1)** | **<0.01** |
| *P for trend* | | | | **<0.01** |
| Patellar | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |
| Grade 1 | **2.46 (1.72, 3.50)** | **<0.01** | **2.39 (1.68, 3.42)** | **<0.01** |
| Grade 2 | **4.89 (3.44, 6.95)** | **<0.01** | **4.52 (3.17, 6.44)** | **<0.01** |
| Grade 3 | **5.78 (4.04, 8.28)** | **<0.01** | **5.22 (3.63, 7.50)** | **<0.01** |
| *P for trend* | | | | **<0.01** |

| | | | | |
|---|---|---|---|---|
| Total | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |
| Grade 1 | **2.52 (1.73, 3.67)** | **<0.01** | **2.46 (1.68, 3.58)** | **<0.01** |
| Grade 2 | **4.20 (2.89, 6.11)** | **<0.01** | **3.89 (2.67, 5.67)** | **<0.01** |
| Grade 3 | **4.98 (3.44, 7.21)** | **<0.01** | **4.31 (2.96, 6.27)** | **<0.01** |
| *P for trend* | | | | **<0.01** |
| ***Any increase in cartilage defects N=402*** | RR | | RR | |
| Medial tibiofemoral | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |
| Grade 1 | 1.15 (0.81, 1.61) | 0.44 | 1.12 (0.79, 1.57) | 0.53 |
| Grade 2 | **1.72 (1.14, 2.59)** | **<0.01** | **1.60 (1.07, 2.40)** | **0.02** |
| Grade 3 | **1.70 (1.14, 2.51)** | **<0.01** | **1.54 (1.01, 2.34)** | **0.04** |
| *P for trend* | | | | **0.01** |
| Lateral tibiofemoral | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |
| Grade 1 | **1.82 (1.12, 2.94)** | **0.02** | **1.81 (1.08, 3.04)** | **0.02** |
| Grade 2 | **2.40 (1.22, 4.69)** | **0.01** | 1.91 (0.90, 4.09) | 0.09 |
| Grade 3 | **2.51 (1.11, 5.67)** | **0.03** | **2.61 (1.20, 5.69)** | **0.02** |
| *P for trend* | | | | **0.03** |
| Patellar | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |
| Grade 1 | 0.84 (0.55, 1.29) | 0.42 | 0.83 (0.54, 1.27) | 0.39 |
| Grade 2 | 1.20 (0.71, 2.02) | 0.50 | 1.16 (0.68, 1.97) | 0.59 |
| Grade 3 | 1.50 (0.79, 2.85) | 0.22 | 1.59 (0.84, 3.03) | 0.15 |
| *P for trend* | | | | 0.20 |
| Total | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |
| Grade 1 | 1.09 (0.84, 1.42) | 0.52 | 1.08 (0.83, 1.41) | 0.57 |
| Grade 2 | **1.38 (1.04, 1.84)** | **0.03** | **1.33 (1.00, 1.77)** | **0.05** |
| Grade 3 | **1.57 (1.20, 2.07)** | **<0.01** | **1.45 (1.09, 1.91)** | **0.01** |
| *P for trend* | | | | **<0.01** |

Dependent variable: baseline presence of (yes vs no) or any increase (yes vs no) in cartilage defects. Independent variable: MRI-detect osteophytes (per grade). OP: osteophytes; PR: prevalence ratio; RR: relative risks; Ref: reference group; *adjusted for age, sex and BMI; ** further adjusted for BMLs, cartilage volume; Significant differences are shown in bold.

In cross-sectional analyses, higher grades of baseline MRI-detected OPs were significantly associated with lower baseline cartilage volume in all compartments, after

adjustment for age, sex, BMI, baseline cartilage defects and BMLs (Table 7.5). In longitudinal analyses, higher grades of baseline MRI-detected OPs were significantly associated with more loss of cartilage volume in total knee, medial and lateral tibiofemoral compartments, after adjustments for covariates (Table 7.5, Figure 1b).

**Table 7. 5** Site-specific associations between baseline MRI osteophytes and baseline/changes in cartilage volume

| | Multivariable* β (95% CI) | p | Multivariable** β (95% CI) | p |
|---|---|---|---|---|
| ***Baseline cartilage volume ($mm^3$)*** | | | | |
| ***N=895*** | | | | |
| Medial tibiofemoral | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |
| Grade 1 | 64.7 (-19.9, 149) | 0.13 | 80.8 (-5.75, 167) | 0.07 |
| Grade 2 | 99.2 (-31.7, 230.1) | 0.14 | 131 (-4.42, 267) | 0.06 |
| Grade 3 | **-229 (-362, -96.5)** | **<0.01** | **-178 (-323, -33.1)** | **0.02** |
| *P for trend* | | | | 0.60 |
| Lateral tibiofemoral | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |
| Grade 1 | 6.20 (-72.1, 84.5) | 0.88 | 14.6 (-65.6, 94.8) | 0.72 |
| Grade 2 | -42.3 (-182, 97.6) | 0.55 | -24.2 (-171, 123) | 0.75 |
| Grade 3 | **-326 (-481, -171)** | **<0.01** | **-296 (-466, -126)** | **<0.01** |
| *P for trend* | | | | **0.03** |
| Patellar | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |
| Grade 1 | **-153 (-276, -30.5)** | **0.01** | **-129 (-251, -7.19)** | **0.04** |
| Grade 2 | **-373 (-531, -214)** | **<0.01** | **-288 (-451, -125)** | **<0.01** |
| Grade 3 | **-737 (-962, -512)** | **<0.01** | **-623 (-854, -392)** | **<0.01** |
| *P for trend* | | | | **<0.01** |
| Total | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |
| Grade 1 | -259 (-523, 5.62) | 0.06 | -217 (-481, 46.4) | 0.11 |
| Grade 2 | -172 (-498, 153) | 0.30 | -43.3 (-374, 287) | 0.80 |
| Grade 3 | **-813 (-1168, -457)** | **<0.01** | **-555 (-940, -171)** | **<0.01** |
| *P for trend* | | | | **0.01** |
| ***Change in cartilage volume (% pa)*** | β | | β | |
| ***N=402*** | | | | |
| Medial tibiofemoral | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |

| | | | | |
|---|---|---|---|---|
| Grade 1 | 0.61 (-0.98, 2.20) | 0.45 | 0.66 (-0.95, 2.27) | 0.42 |
| Grade 2 | **-4.88 (-7.57, -2.19)** | **<0.01** | **-5.0 (-7.79, -2.21)** | **<0.01** |
| Grade 3 | **-3.13 (-6.10, -0.17)** | **0.04** | **-3.25 (-6.43, -0.06)** | **0.05** |
| *P for trend* | | | | **0.01** |
| | | | | |
| Lateral tibiofemoral | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |
| Grade 1 | 0.93 (-0.16, 2.03) | 0.09 | 0.98 (-0.13, 2.09) | 0.08 |
| Grade 2 | -1.17 (-3.34, 1.00) | 0.29 | -1.07 (-3.30, 1.17) | 0.35 |
| Grade 3 | **-5.96 (-8.36, -3.55)** | **<0.01** | **-5.95 (-8.53, -3.37)** | **<0.01** |
| *P for trend* | | | | **<0.01** |
| | | | | |
| Patellar | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |
| Grade 1 | -0.21 (-1.51, 1.08) | 0.75 | -0.16 (-1.47, 1.14) | 0.81 |
| Grade 2 | -0.49 (-2.16, 1.19) | 0.57 | -0.25 (-1.98, 1.48) | 0.78 |
| Grade 3 | -0.90 (-3.12, 1.33) | 0.43 | -0.49 (-2.85, 1.87) | 0.68 |
| *P for trend* | | | | 0.68 |
| | | | | |
| Total | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |
| Grade 1 | -0.03 (-0.72, 0.66) | 0.93 | -0.01 (-0.69, 0.70) | 0.98 |
| Grade 2 | **-1.17 (-2.01, -0.33)** | **<0.01** | **-1.10 (-1.94, -0.25)** | **0.01** |
| Grade 3 | **-0.90 (-1.80, -0.01)** | **0.05** | -0.78 (-1.75, 0.20) | 0.12 |
| *P for trend* | | | | **<0.01** |

Dependent variable: baseline or change in cartilage volume. Independent variable: MRI-detect osteophytes (per grade). OP: osteophytes; PR: prevalence ratio; RR: relative risks; Ref: reference group; *adjusted for age, sex and BMI; ** further adjusted for BMLs and cartilage defects; Significant differences are shown in bold.

Similarly, higher grades of baseline MRI-detected OPs were significantly and site-specifically associated with greater prevelances of baseline BMLs at all compartments, after adjustment for age, sex, baseline cartilage volume and cartilage defects (Table 7.6). The longitudinal associations between baseline grades of MRI-detected OPs and any increase in BMLs at total knee, medial and lateral tibiofemoral compartments were also significant in multivariable analyses (Table 7.6, Figure 7.1c). Sensitivity analyses showed that these significant associations between MRI-detected OPs and structural abnormalities were similar in those without X-ray OPs (data not shown).

**Table 7. 6** Site-specific associations between baseline MRI osteophytes and baseline/increases in BMLs

| | Multivariable* PR (95% CI) | p | Multivariable** PR (95% CI) | p |
|---|---|---|---|---|
| ***Presence of BMLs at baseline N=895*** | | | | |
| Medial tibiofemoral | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |
| Grade 1 | **1.51 (1.08, 2.12)** | **0.02** | 1.37 (0.97, 1.93) | 0.08 |
| Grade 2 | **2.07 (1.35, 3.18)** | **<0.01** | **1.72 (1.11, 2.68)** | **0.02** |
| Grade 3 | **3.85 (2.89, 5.13)** | **<0.01** | **2.74 (1.96, 3.84)** | **<0.01** |
| *P for trend* | | | | **<0.01** |
| Lateral tibiofemoral | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |
| Grade 1 | 1.31 (0.93, 1.84) | 0.12 | 1.09 (0.75, 1.57) | 0.57 |
| Grade 2 | **2.29 (1.44, 3.63)** | **<0.01** | **1.89 (1.11, 3.21)** | **0.02** |
| Grade 3 | **3.62 (2.39, 5.49)** | **<0.01** | **2.10 (1.32, 3.35)** | **<0.01** |
| *P for trend* | | | | **<0.01** |
| Patellar | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |
| Grade 1 | **1.66 (1.08, 2.56)** | **0.02** | **1.72 (1.08, 2.74)** | **0.02** |
| Grade 2 | **2.87 (1.81, 4.57)** | **<0.01** | **2.87 (1.75, 4.70)** | **<0.01** |
| Grade 3 | **2.42 (1.31, 4.47)** | **<0.01** | **2.02 (1.01, 4.05)** | **0.05** |
| *P for trend* | | | | **<0.01** |
| Total | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |
| Grade 1 | **1.34 (1.05, 1.70)** | **0.02** | 1.21 (0.95, 1.54) | 0.13 |
| Grade 2 | **2.06 (1.42, 2.99)** | **<0.01** | **1.78 (1.18, 2.69)** | **<0.01** |
| Grade 3 | **2.93 (2.04, 4.23)** | **<0.01** | **1.88 (1.24, 2.84)** | **<0.01** |
| *P for trend* | | | | **<0.01** |
| ***Any increase in BMLs N=402*** | RR | | RR | |
| Medial tibiofemoral | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |
| Grade 1 | 1.27 (0.70, 2.30) | 0.43 | 1.14 (0.62, 2.10) | 0.67 |
| Grade 2 | **2.79 (1.42, 5.48)** | **<0.01** | 1.92 (0.96, 3.84) | 0.07 |
| Grade 3 | **3.64 (2.00, 6.60)** | **<0.01** | **2.08 (1.12, 3.86)** | **0.02** |
| *P for trend* | | | | **<0.01** |
| Lateral tibiofemoral | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |

| | | | | |
|---|---|---|---|---|
| Grade 1 | 0.97 (0.61, 1.54) | 0.88 | 0.86 (0.54, 1.37) | 0.52 |
| Grade 2 | 1.57 (0.81, 3.07) | 0.18 | 1.02 (0.50, 2.09) | 0.95 |
| Grade 3 | **3.19 (1.98, 5.14)** | **<0.01** | **2.04 (1.14, 3.65)** | **0.02** |
| *P for trend* | | | | **<0.01** |
| | | | | |
| Patellar | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |
| Grade 1 | 1.16 (0.64, 2.12) | 0.62 | 1.11 (0.59, 2.08) | 0.39 |
| Grade 2 | 1.19 (0.58, 2.42) | 0.64 | 1.32 (0.60, 2.91) | 0.50 |
| Grade 3 | 1.71 (0.74, 3.93) | 0.21 | 2.22 (0.90, 5.49) | 0.08 |
| *P for trend* | | | | 0.35 |
| | | | | |
| Total | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |
| Grade 1 | 0.91 (0.62, 1.33) | 0.63 | 0.88 (0.59, 1.30) | 0.57 |
| Grade 2 | 1.48 (0.93, 2.36) | 0.10 | 1.11 (0.64, 1.92) | 0.71 |
| Grade 3 | **2.53 (1.78, 3.61)** | **<0.01** | **1.56 (1.03, 2.40)** | **0.04** |
| *P for trend* | | | | **<0.01** |

Dependent variable: baseline presence (yes vs no) of or any increase (yes vs no) in BMLs. Independent variable: MRI-detect osteophytes (per grade). BMLs: bone marrow lesions; OP: osteophytes; PR: prevalence ratio; RR: relative risks; Ref: reference group; *adjusted for age, sex and BMI; ** further adjusted for cartilage defects and cartilage volume. Significant differences are shown in bold.

### 7.3.4 Associations with WOMAC pain

Table 7.7 described the associations between baseline MRI-detected OPs and the presence of or any increase in WOMAC knee pain. Participants who had higher grades of baseline MRI-detected OPs, particularly in grade 2 and 3, had higher prevalence of WOMAC pain and greater risks of worsening WOMAC pain scores over 2.6 and 5 years, before and after adjustments for age, sex, BMI (Table 7.4). Figure 7.1d shows significant associations between baseline MRI-detected OPs in different compartments and worsening total WOMAC knee pain over 5 years. The cross-sectional associations remained significant after further adjustment for baseline cartilage defects and BMLs; however, longitudinal associations were no longer statistically significant after further adjustments (Table 7.7).

**Table 7. 7** Cross-sectional and longitudinal associations between baseline MRI-detected osteophytes and baseline and increases in WOMAC knee pain

| | Multivariable* PR (95% CI) | p | Multivariable** PR (95% CI) | p |
|---|---|---|---|---|
| ***Presence of knee pain at baseline*** | | | | |
| ***N=892*** | | | | |
| Total MRI-detected OPs | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |
| Grade 1 | 1.05 (0.84, 1.30) | 0.68 | 1.05 (0.84, 1.31) | 0.64 |
| Grade 2 | **1.30 (1.03, 1.66)** | **0.03** | **1.31 (1.03, 1.66)** | **0.03** |
| Grade 3 | **1.80 (1.44, 2.26)** | **<0.01** | **1.79 (1.41, 2.27)** | **<0.01** |
| *P for trend* | | | | **<0.01** |
| ***Increase in WOMAC knee pain over 2.6 years*** | | | | |
| ***N=787*** | RR (95% CI) | p | RR (95% CI) | p |
| Total MRI-detected OPs | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |
| Grade 1 | 1.20 (0.78, 1.85) | 0.40 | 1.16 (0.75, 1.80) | 0.50 |
| Grade 2 | 1.07 (0.63, 1.83) | 0.80 | 0.95 (0.55, 1.66) | 0.87 |
| Grade 3 | **1.67 (1.00, 2.78)** | **0.05** | 1.35 (0.77, 2.37) | 0.30 |
| *P for trend* | | | | **0.03** |
| ***Increase in WOMAC knee pain over 5 years*** | | | | |
| ***N=690*** | | | | |
| Total MRI-detected OPs | | | | |
| Grade 0 | *Ref.* | | *Ref.* | |
| Grade 1 | 0.90 (0.65, 1.26) | 0.55 | 0.85 (0.61, 1.20) | 0.37 |
| Grade 2 | 1.20 (0.77, 1.88) | 0.41 | 1.01 (0.63, 1.60) | 0.98 |
| Grade 3 | **1.63 (1.08, 2.45)** | **0.02** | 1.24 (0.78, 1.97) | 0.37 |
| *P for trend* | | | | **0.04** |

Dependent variable: baseline and increases in WOMAC knee pain (yes or no). Independent variable: MRI-detect total knee osteophytes. OP: osteophytes; PR: prevalence ratio; *adjusted for age, sex and BMI. ** Further adjustment for baseline cartilage defects and BMLs. Significant differences are shown in bold.

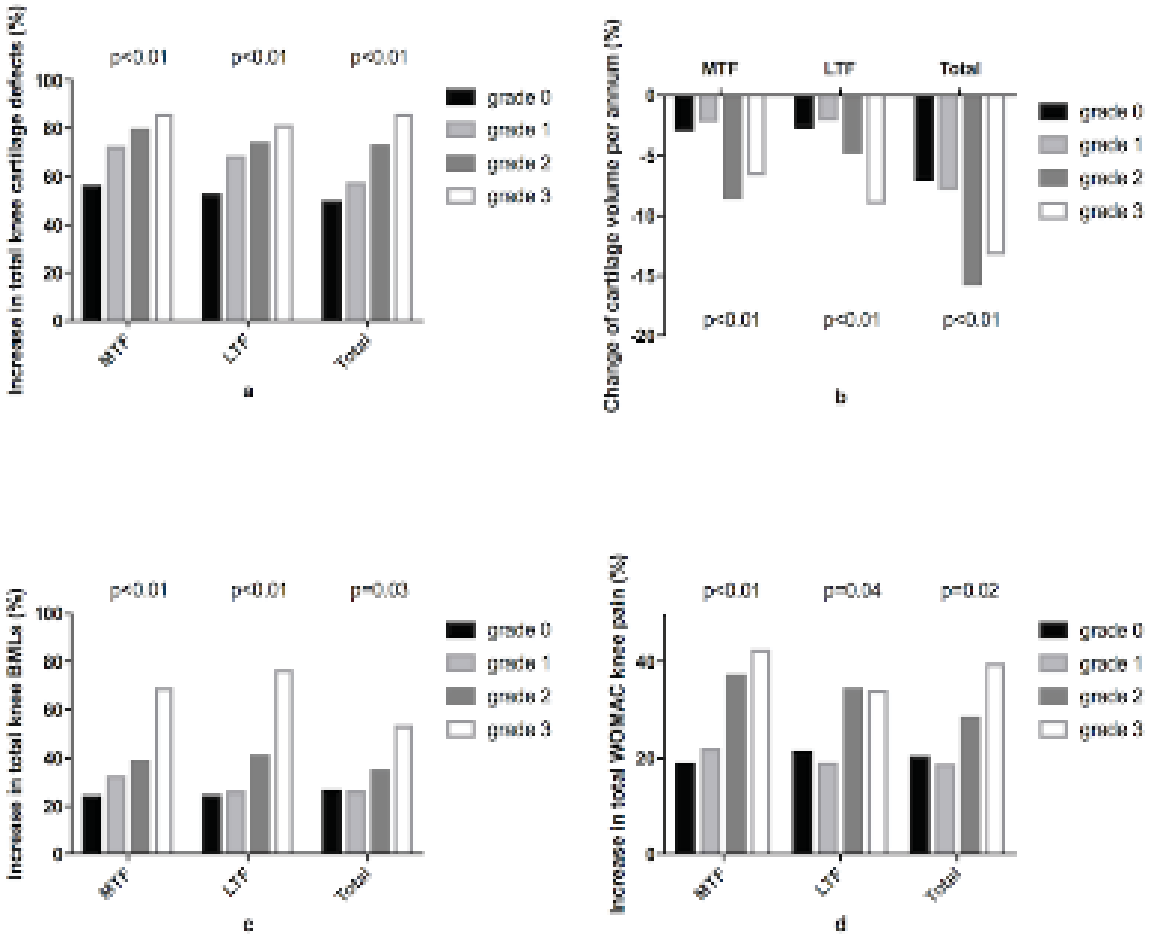



**Figure 7. 1** Association of baseline MRI-detected OPs with increases in total knee cartilage defects

(a), changes in cartilage volume per annum (%) (b), increases in total knee BMLs (c), and increases in total WOMAC knee pain over 5 years (d). MTF: medial tibiofemoral; LTF: lateral tibiofemoral. p values were for trends at different compartments after adjustment for baseline age, sex and body mass index.

## 7.4 Discussion

In our study, OPs detected on MRI were much more common than OPs visible on conventional radiographs, as expected. MRI-detected OPs were associated with knee structural abnormalities both cross-sectionally and longitudinally. Significant

associations between MRI-detected OPs and WOMAC knee pain were also found but these were largely dependent of knee structural abnormalities. These results suggest that MRI-detected OPs may be an early marker of the disease process in knee OA.

Conventional radiographs are known to be relatively insensitive to the structural changes of OA [107], in part because of their inability to detect three-dimensional (3D) joint structures [107], and inadequate visualization of early and central OPs. One study reported that prevalence of MRI-defined OP was 72% among middle-aged women [110]. Another study looked at the prevalence of MRI-depicted abnormalities in knees without radiographic evidence of OA and found that OPs were the most common abnormality, being present in 74% of 710 participants [107]. Our data also showed a much higher prevalence of MRI-detected OPs in older adults than the prevalence of radiographially-detected OPs (85% vs 10%). MRI-detected OPs also had high reliabilities than radiographically-detected OPs. These findings suggest that MRI is far more sensitive and reliable than X-ray to detect osteophytes and our data suggest these OPs have clinical relevance.

*Structural changes*

Significant cross-sectional associations between MRI-identified OPs and radiographic severity of knee OA were reported among middle-aged women [63]. Another cross-sectional study revealed that MRI-detected OPs was only weakly associated with synovitis or joint effusion [270]. There are only two longitudinal studies so far, which reported inconsistent results [110, 112]. The first did not reveal any significant associations between MRI- defined OPs and knee structural progression [110]. The second was a nested case-control study reporting that subjects with 6 or more locations affected by OPs had 4.4-fold the odds of being both radiographic and pain progression compared with 0-2 locations affected [112]. Our current study reported positive, consistent and independent associations between MRI-detected OPs and changes in knee cartilage and bone abnormalities both cross-sectionally and longitudinally in a community-based older population. These associations remained unchanged after those with X-ray detected OPs were excluded. Although the underlying mechanisms are

unable to be determined in this study, our findings imply that MRI-detected osteophytes could be a precursor of cartilage degradation and BMLs.

*Pain*

The association between OPs and knee pain is still controversial. One cohort study reported that increasing baseline OP size was associated with increasing WOMAC pain severity score [110] in a middle-aged female population (n=363). Another cross-sectional study reported a significant association between presence of OPs and knee pain among symptomatic OA patients (n=368) only when OPs were located in the patellofemoral compartment or when more than four OPs (any grade) were present anywhere in the knee [44]. In contrast, Link et al [111] reported that MRI-defined OPs were not associated with clinical findings as assessed with the WOMAC scores in patients with varying degrees of OA (n=50). A recent systematic review concluded that there was limited level of evidence for associations between MRI-detected OPs and knee pain [82]. Compared to these previous studies, our study was performed in a general population with a large sample size (n=837) and revealed that there was a significant association between MRI-detected OPs and total WOMAC knee pain cross-sectionally, independent of knee structural abnormalities. MRI-detected OPs were also significantly associated with changes in knee pain over 2.6 years and 5 years, but these associations became non-significant after adjustment for cartilage defects and BMLs, indicating MRI-detected OPs may cause OA symptoms through other structural changes. Our extensive analyses showed that MRI-detected OP at medial compartment had the most prominently and consistently significant associations with WOMAC knee pain changes than others (data not shown). This is reasonable as medial compartment has the most frequently osteoarthritic changes among all the compartments [271].

Strengths of this study included the random selection of participants for the cohort from the community, with a large sample size and both structural and symptomatic measurements. Our results have good external validity, as they can be generalizable to all white older adults in the population. Study limitations included the unavailability of follow-up MRI scans in 489 participants due to decommissioning of MRI scanner.

However, the current study sample is similar to the remainder of the cohort in terms of demographic factors, ROA, baseline cartilage volume, defects and BMLs. Second, we did not perform MRI scan at year 5 so were not able to assess the associations with changes in knee structures over 5 years. Last, different semi-quantitative scoring systems were used for OPs, cartilage defects and BMLs which may influence results; however, given all measures were highly reproducible, this is considered unlikely.

In conclusion, MRI-detected OPs are common and appear to be clinically relevant to knee structural changes in older adults.

# Chapter 8

# Association between MRI-detected early osteophytes and knee structure in older adults: a population-based cohort study

This manuscript has been submitted for publication. The text of this chapter is the same as the submitted version. Thus, there is some repetition of the methods.

## 8.1 Introduction

Knee osteoarthritis (OA) is a leading cause of pain and disability [2]. Symptomatic knee OA is estimated to occur in 10% of men and 13% of women aged 60 years or older in the United State [40]. Although osteophytes (OPs) have long been viewed as a defining structural feature of knee OA [272] and a fundamental sign of disease incidence and progression [273], the correlation between OPs and clinical feature is weak at best [47, 103], and change in symptoms is poorly predicted by baseline radiographic OPs [264].

In an observational study, knee pain was reported by 1004 subjects, only 15% of whom had radiographic grade 2 to 4 changes of OA [274]. The discrepancy between clinical and radiographic OA may be due to the inherent limitations of conventional radiography as an imaging tool [275]. Many OA features cannot be detected using radiography and some pre-radiographic OA features are missed using radiographic assessment. A recent study revealed that about 90% of radiographically normal knees had one or more OA-related features on MRI, and MRI-detected OP is the most common abnormality among these features [107]. An observational study has reported that prevalence of MRI-detected OPs is 72% among middle-aged women [110] and another study reports 74% MRI-detected OPs in 710 knees without radiographic evidence of OA [107]. In contrast, the prevalence of radiographic OPs was approximately 10% in a generally older population (mean age 61 years) [108].

Given that radiography fails to detect a large proportion of OPs which can be detected on MRI, there would be a large number of OA patients who have MRI-detected early OPs (MRI-OPs) but are misclassified as normal. Moreover, they represent different stages of OA process. To date, the relevance of MRI-OPs for the development of structural and clinical abnormalities is uncertain. We hypothesized that MRI-OPs that are detected only by MRI can serve as a biomarker in identifying patients at a high risk of osteoarthritic progression. The aim of this population-based cohort study, therefore, was to describe the prevalence of MRI-OPs in older adults and the longitudinal associations with knee pain and structural abnormalities.

## 8.2 Material and methods

### 8.2.1 Subjects

These analyses use data from the Tasmania Older Adult Cohort (TASOAC) Study, a population-based, ongoing, prospective study which was designed to identify the environmental, genetic, biochemical factors associated with the development and progression of OA at multiple sites. Participants between 50 and 80 years old were randomly selected from the electoral roll in Southern Tasmania (population 229, 000) using sex-stratified random sampling (response rate 57%). Participants were excluded if they were institutionalised or had contraindications to MRI. The Southern Tasmania Health and Medical Human Research Ethics Committee approved the study, and written informed consent was obtained from all participants. Baseline examinations were taken between February 2002 and September 2004, and follow-up measures were taken at approximately 2.6 and 5.1 years later. This study consisted of 837 participants who had knee MRI and radiographic scans at baseline.

### 8.2.2 Magnetic Resonance Imaging

MRI scans of the right knees were performed on two occasions and imaged in the sagittal plane on a 1.5-T whole body magnetic resonance unit (Picker, Cleveland, OH) using a commercial transmit-receive extremity coil. The image sequences used are listed as follows: (1) a T1-weighted fat saturation 3D gradient recall acquisition in the steady state; flip angle 30°; repetition time 31 ms; echo time 6.71 ms; field of view 16 cm; 60 partitions; 512×512 matrix; acquisition time 11 min 56 s; one acquisition. Sagittal images were obtained at a partition thickness of 1.5 mm and an in-plane resolution of 0.31×0.31 (512×512 pixels). (2) a T2-weighted fat saturation 3-D fast spin echo, flip angle 90, repetition time 3067 ms, echo time 112 ms, field of view 16 cm, 15 partitions, 228x256-pixel matrix; sagittal images were obtained at a partition thickness of 4 mm with a between-slices gap of 0.5 to 1.0 mm. The image database was transferred to an independent computer workstation using the software program Osirix (University of Geneva, Geneva, Switzerland) as previously described [116, 191].

### 8.2.3 MRI-detected osteophytes

MRI-detected OPs were measured by ZZ according to the Knee Osteoarthritis Scoring System (KOSS) [196] where OPs are defined as focal bony excrescences, seen on sagittal, axial or coronal images, extending from a cortical surface. OPs were measured using the following scale. Grade 0, absent; grade 1, minimal (<3mm); grade 2, moderate (3-5 mm); grade 3, severe (>5 mm) [196]. Size was measured from the base (distinguished from that of adjacent articular cartilage with a normal MRI appearance) to the tip of the OP [188] at each of the following 14 sites: the anterior (a), central weight bearing (c) and posterior (p) margins of the femoral condyles(medial and lateral), and tibial plateaus (medial and lateral), and the medial (M) and lateral (L) margins of the patella [125]. The worst score of each individual site in the relevant compartment (or whole knee) was regarded as the OP score in that compartment (or whole knee). MRI-detected OP score of ≥1 was considered as OP present. MRI-detected OPs were remeasured in 40 randomly selected participants with four weeks interval by ZZ and WH to calculate intra-observer and inter-observer reliabilities. Intra-observer reliability (expressed as intraclass correlation coefficients, ICCs) was 0.94-0.97 and inter-observer reliability was 0.90-0.96.

### 8.2.4 Cartilage defects

Cartilage defects were graded by CD at medial tibial, lateral tibial, medial femoral, lateral femoral and patellar regions as previously described [126, 129] as follows. Grade 0, normal cartilage; grade 1, focal blistering and low-signal intensity change with an intact surface and bottom; grade 2, irregularities on the surface or bottom and loss of thickness of less than 50%; grade 3, deep ulceration with loss of thickness of more than 50%; grade 4, full thickness cartilage loss with exposure of subchondral bone [126]. The worst score of each individual site in the relevant compartment (or whole knee) was regarded as the cartilage defect score in that compartment (or whole knee). The presence of cartilage defects was defined as a cartilage defect score of ≥2 at any site (medial tibial, lateral tibial, medial femoral or lateral femoral). An increase in cartilage defects was defined as a change in cartilage defects of ≥1. Intra-observer reliability was 0.89-0.94 and inter-observer reliability was 0.85-0.93 [126].

### 8.2.5 Cartilage volume

Knee cartilage volume was measured on T1-weighted images by a single trained observer at baseline as previously described [193, 194]. The volumes of individual cartilage plates (medial tibial, lateral tibial, medial femoral, lateral femoral and patellar) were isolated from the total volume by manually drawing disarticulation contours around the cartilage boundaries on a section by section basis. These data were resampled by means of bilinear and cubic interpolation (area of 312×312) um and 1.5 mm thickness, continuous sections) for the final 3-dimensional rendering. Changes in cartilage volume were calculated as: percentage change per annum= [(follow-up volume – baseline volume)/baseline cartilage volume]/time between 2 scans in years × 100. The coefficients of variation (CVs) for cartilage volume measures were 2.1% to 2.6% [193, 194].

### 8.2.6 Bone marrow lesions

Subchondral bone marrow lesions (BMLs) were defined as discrete areas of increased signal adjacent to the subcortical bone on T2-weighted MRI and scored at medial tibial, lateral tibial, medial femoral, lateral femoral, and patellar regions, using a modified version of Whole-Organ Magnetic Resonance Imaging Score (WORMS). Grade 0, absence of BML; grade 1, area smaller than 25% of the region; grade 2, area between 25% to 50% of the region; grade 3, area larger than 50% of the region [125]. The worst score of each individual site in the relevant compartment (or whole knee) was regarded as the BML score in that compartment (or whole knee). The presence of BMLs was defined as a BML score of ≥1 at any site (medial tibial, lateral tibial, medial femoral or lateral femoral). An increase in BMLs was defined as a change in BMLs of ≥1. The intraclass correlation coefficients (ICCs) for intra-observer reliability were 0.89-0.96 [205]. The inter-observer reliability of this BML scoring system was assessed by randomly selecting 40 subjects with BMLs and having their MRI scans re-read by another observer. The ICCs for inter-observer reliability were also excellent (0.73-0.95) [71, 195].

### 8.2.7 X-ray assessment

A standing anteroposterior semiflexed view of the right knee with 15° of fixed knee flexion was performed in all subject at baseline. Joint space narrowing (JSN) and radiographic osteophytes (OPs) were scored at each site of medial tibia, medial femur, lateral tibia and lateral femur on a scale of 0-3 (0=normal, 3= severe) according to the Osteoarthritis Research Society International (OARSI) atlas developed by Altman et al. [31]. Medial tibiofemoral (femoral and tibial combined) X-ray-detected OP and lateral tibiofemoral X-ray-detected OP were the worst scores of the four sites (medial tibia, medial femur, lateral tibia and lateral femur). The total X-ray-detected OP score was the worst score of all compartments of the knee. The presence of X-ray-detected OP was defined as X-ray-detected OP scores of ≥ 1 in the specific compartment. Due to lack of skyline view of radiographs, patellar radiographic OPs were not obtained. The presence of radiographic OA (ROA) was defined as any score of ≥ 1 (JSN or OP). Each score was determined by two readers who simultaneously assessed the radiograph with immediate reference to the atlas. Intraobserver repeatability was tested in 40 subjects one month apart with ICCs of 0.65-0.85 [23].

### 8.2.8 WOMAC pain assessment

Knee pain was assessed using the Western Ontario McMaster Osteoarthritis Index (WOMAC) [204] at baseline and 5 years later using a 10-point scale from 0 (no pain) to 9 (most severe pain). The 5 subscales (walking on flat surface, going up/down stairs, at night, sitting/lying and standing upright) were assessed separately and summed to create a total pain score (0 to 45). Change in knee pain score was calculated as follow-up value - baseline value. The presence of knee pain was defined as total WOMAC pain score of 1 or greater [268]. Worsening knee pain was defined as a change in WOMAC pain score of 1 or greater. Regular nonsteroridal anti-inflammatory drugs (NSAIDs) use in most days (>15 days) of the last month at baseline were recorded by questionnaire.

### 8.2.9 Anthropometrics

Height was measured to the nearest 0.1 cm (with shoes, and headgear removed) using a stadiometer. Weight was measured to the nearest 0.1 kg (with shoes, socks, and bulky

clothing removed) by using a single pair of electronic scales (Delta Model 707, Seca, Hamburg, Germany) that were calibrated using a known weight at the beginning of each clinic. Body mass index (BMI, weight (kg)/height ($m^2$)) was also calculated.

### 8.2.10 Data analysis

One-way analysis of variance or $\chi^2$ test were used to compare means or proportions among participants with no OPs (no X-ray or MRI OPs), MRI-OPs (only MRI OPs, not detected by X-ray) and established-OPs (both X-ray and MRI OPs). Multivariable linear regression analyses were used to examine the compartment-specific associations between different phenotypes of OP (independent variables) and knee cartilage volume change (dependent variable), with age, sex, BMI, cartilage defects and BMLs as covariates. Multivariable log binominal regression analyses were used to assess compartment-specific associations between different phenotypes of OP (independent variables) and increases in cartilage defects /BMLs (dependent variables); multivariable linear regression analyses were also used to evaluate longitudinal associations between OP phenotypes and change of total WOMAC knee pain over 5 years, both after adjustment for potential confounders. All statistical analyses were performed on Stata version 12.0 for Windows (StataCorp, College Station, TX, USA)

A *p*-value < 0.05 (2-tailed) or a 95% confidence interval (CI) not including the null point (for linear regression) or 1 (for log binominal regression) was considered as statistical significance.

## 8.3 Results

### 8.3.1 Characteristics of study sample

Of the 837 participants, 422 (50%) had MRI-OPs, 333 (40%) had no-OPs and 80 (10%) had established-OPs in the total tibiofemoral compartment of knee. In medial tibiofemoral compartment, 205 (24%) had MRI-OPs, and in lateral tibiofemoral compartment 446 (53%) had MRI-OPs (Table 8.1). 2 cases had OPs only seen on radiographs. We ignored this group as the sample was too small to do any proper analyses. In total 422 knees that had MRI-OPs in total tibiofemoral compartment, 155

knees had the highest OP score based on tibia, 340 knees based on femora, and 73 knees had equivalent highest OP score based on two sites. There were no significant differences in baseline demographics, cartilage defects, BMLs, or cartilage volume between the subjects who were included in the present study and the rest of the cohort (data not shown). Due to lack of skyline view of radiographs, MRI-OPs at patellofemoral compartment was not investigated in current study. Follow-up MRI scans were only available in 395 out of 837 participants. However, there were no significant differences in baseline demographics, cartilage defects, BMLs, or cartilage volume between participants who were included in the present study and those who did not have follow-up MRI scans (data not shown).

The baseline characteristics of the participants are shown in Table 8.2. Over the observational period, 83%, 69%, and 53% of participants had persistent MRI-detected OP scores and 17%, 30%, and 46% of subjects had increased MRI-detected OP scores in the medial tibiofemoral compartment, lateral tibiofemoral compartment, and total knee compartment, respectively. Change in MRI-detected OP scores were significant associated with increases in cartilage defects, BMLs before and after adjusted for age, sex, BMI and baseline structural abnormalities (data not shown). At baseline, subjects with no-OPs, established-OPs and MRI-OPs were significant different in terms of age ($p<0.01$), body weight ($p<0.01$), BMI ($p<0.01$), tibial bone area ($p<0.01$), prevalence of JSN ($p<0.01$), cartilage defects and BMLs ($p<0.01$), and total cartilage defect and BML scores ($p<0.01$). Subjects with no, MRI-, and established-OPs were similar in terms of baseline cartilage volume and female proportion.

**Table 8. 1** Frequencies of OP types detected by x-ray and MRI in the studied sample

| | Total TF | | | MTF | | | LTF | | |
|---|---|---|---|---|---|---|---|---|---|
| | x-ray OPs | MRI OPs | n | x-ray OPs | MRI OPs | n | x-rays OP | MRI OPs | n |
| No OPs | N | N | 333 | N | N | 571 | N | N | 358 |
| MRI-OPs | N | Y | 422 | N | Y | 205 | N | Y | 446 |
| | Y | N | 2 | Y | N | 2 | Y | N | 0 |
| Established-OPs | Y | Y | 80 | Y | Y | 59 | Y | Y | 33 |
| Total | | | 837 | | | 837 | | | 837 |

OP: osteophytes; TF: tibiofemoral; MTF: medial tibiofemoral; LTF: lateral tibiofemoral; Y means with x-ray OP or MRI OP, N means without x-ray OP or MRI OP.

**Table 8. 2** Baseline characteristics of participants

| | No OP | MRI-OPs | Established-OPs | Total Sample |
|---|---|---|---|---|
| | N=333 | N=422 | N=80 | N=834 |
| Age (year) | **62±7.2** | **62±7.5** | **66±6.8** | 62±7.4 |
| Female sex (%) | 53 | 48 | 48 | 50 |
| Weight (kg) | **75±13** | **79±15** | **86±15** | 78±15 |
| BMI (kg/m²) | **27±4.2** | **28±4.4** | **31±6.2** | 28±4.7 |
| Total tibial bone area (cm²) | **32±1.5** | **33±0.5** | **35±0.6** | 33±5.0 |
| Any joint space narrowing (%) | **52** | **58** | **95** | 59 |
| Any joint space narrowing score (n) | | | | |
| 0 | 160 | 178 | 4 | 342 |
| 1 | 136 | 188 | 18 | 343 |
| 2 | 34 | 45 | 35 | 115 |
| 3 | 3 | 11 | 23 | 37 |
| Total cartilage defects score (0-4) | **1.4±0.7** | **2.0±0.9** | **3.0±1.0** | 1.8±0.9 |
| Total BML score (0-3) | **0.27±0.49** | **0.49±0.73** | **1.1±1.0** | 0.47±0.71 |
| Total tibial cartilage volume (ml) | 5.0±1.2 | 5.1±1.2 | 4.8±1.3 | 5.1±1.2 |
| Any cartilage defects (%) | **32** | **61** | **93** | 53 |
| Any BMLs present (%) | **25** | **37** | **68** | 34 |
| Knee pain present (%) | **47** | **50** | **76** | 51 |
| Total WOMAC score (0-45) | **3.3±6.3** | **3.1±5.6** | **6.5±7.5** | 3.5±6.1 |

One-way analysis of variance was used for differences between groups, and $\chi 2$ tests were used for proportions (percentages). Mean ± SD except for percentages. Significant differences are shown in bold. OPs: osteophytes; BMI: body mass index; BML: bone marrow lesions.

### 8.3.2 Associations with cartilage defects

Figure 8.1a shows a dose-response relationship between baseline OP phenotypes and increases in knee cartilage defects in different knee compartments. Compared to knees with no-OPs, knees with MRI-OPs were associated with a greater risk of increased cartilage defect scores in medial tibiofemoral, lateral tibiofemoral and total tibiofemoral compartments, after adjustment for age, sex, BMI, baseline cartilage volume, JSN and BMLs in the same compartments. Similarly, knees with established-OPs had greater risk of increased cartilage defect scores in total tibiofemoral and medial tibiofemoral, but not in lateral tibiofemoral, compartment, after adjustment for relevant covariates and the effect sizes were larger than MRI-OPs group (Table 8.3).

**Table 8. 3** Longitudinal associations of OP phenotype status and changes/increases in total knee structure in 2.6 years

| | **Increases in Cartilage Defects** | | **Cartilage Volume changes (p.a)** | | **Increases in BMLs** | |
|---|---|---|---|---|---|---|
| | **Adjusted*** | **Adjusted**** | **Adjusted*** | **Adjusted**** | **Adjusted*** | **Adjusted**** |
| | RR (95% CI) | RR (95% CI) | β (95% CI) | β (95% CI) | RR (95% CI) | RR (95% CI) |
| ***OP phenotypes n=395*** | | | | | | |
| ***Total tibiofemoral*** | | | | | | |
| No OPs (n=152) | *Ref.* | *Ref.* | *Ref.* | *Ref.* | *Ref.* | *Ref.* |
| MRI-OPs (n=211) | **1.46 (1.17, 1.83)** | **1.37 (1.07, 1.74)** | -0.02 (-0.95, 0.90) | 0.05 (-0.90, 0.99) | 1.07 (0.76, 1.52) | 0.89 (0.62, 1.27) |
| Established-OPs (n=32) | **1.93 (1.49, 2.51)** | **1.66 (1.15, 2.40)** | **-2.27 (-4.02, -0.17)** | **-2.02 (-3.86, -0.17)** | **2.50 (1.70, 3.69)** | **1.88 (1.22, 2.89)** |
| P for trend | | **p<0.01** | | **p=0.03** | | **p<0.01** |
| ***Medial tibiofemoral*** | | | | | | |
| No OPs (n=259) | *Ref.* | *Ref.* | *Ref.* | *Ref.* | *Ref.* | *Ref.* |
| MRI-OPs (n=111) | **1.31 (1.12, 1.53)** | **1.26 (1.08, 1.48)** | **-0.56(-1.09, -0.04)** | -0.54 (-1.08, 0.01) | **1.52 (1.10, 2.11)** | **1.51 (1.08, 2.11)** |
| Established-OPs (n=24) | **1.64 (1.41, 1.90)** | **1.49 (1.26, 1.75)** | -0.79 (-1.83, 0.26) | -0.47 (-1.57, 0.63) | **2.29 (1.48, 3.56)** | **2.16 (1.36, 3.45)** |
| P for trend | | **p<0.01** | | **p<0.01** | | **p<0.01** |
| ***Lateral tibiofemoral*** | | | | | | |
| No OPs (n=165) | *Ref.* | *Ref.* | *Ref.* | *Ref.* | *Ref.* | *Ref.* |
| MRI-OPs (n=219) | **1.33 (1.13, 1.57)** | **1.28 (1.08, 1.51)** | -0.01 (-1.12, 1.14) | -0.14 (-0.64, 0.37) | 1.23 (0.88, 1.71) | 0.97 (0.63, 1.50) |
| Established-OPs (n=11) | **1.50 (1.08, 2.09)** | **1.44 (1.05, 1.97)** | **-5.93 (-10.2, -1.70)** | **-5.41 (-9.68, -1.13)** | **2.60 (1.59, 4.26)** | **1.88 (1.18, 3.00)** |
| P for trend | | **p=0.01** | | p=0.08 | | **p=0.01** |

OP: osteophytes; p.a, percentage per annual; Results of this table are generated from a linear regression or log binominal regression model. *Adjusted for age, sex and BMI;

** Further adjusted for cartilage volume, cartilage defects, JSN and BMLs (excluded the outcome structures); Significant differences are showed in bold.

### 8.3.3 Associations with cartilage volume

Figure 8.1b shows significant associations of baseline OP phenotypes with changes of total cartilage volume in different compartments. Comparing with knees with no-OPs, knees with MRI-OPs had significantly greater loss of cartilage volume over 2.6 years in medial tibiofemoral compartment, after adjustments for age, sex and BMI, cartilage defects and BMLs in the same compartments, but became statistically non-significant after further adjustment for JSN. Associations between MRI-OPs and cartilage loss in total tibiofemoral and lateral tibiofemoral compartments were not significant. Established-OPs were significantly associated with loss of knee cartilage volume over 2.6 years in total tibiofemoral and lateral tibiofemoral compartments, after adjustment for age, sex and BMI, these significant associations persisted after further adjustment for cartilage defects, JSN and BMLs in the same compartments. No significant associations were found between established-OPs and cartilage volume loss in medial tibiofemoral compartments (Table 8.3).

### 8.3.4 Associations with BMLs

Figure 8.1c showed significant associations between baseline OP phenotypes and increases in total knee BMLs in different compartments. Comparing with no-OPs knees, knees with MRI-OPs had higher risks of having increased medial tibiofemoral BMLs over 2.6 years, after adjustment for age, sex and BMI, and remained significant after further adjustment for cartilage volume, JSN and cartilage defects. MRI-OPs were not significantly associated with increases in BMLs in the total tibiofemoral and lateral tibiofemoral compartments. Knees with established-OPs had significantly higher risks of increased knee BMLs over 2.6 years in total tibiofemoral, medial tibiofemoral and lateral tibiofemoral compartments after adjustment for age, sex and BMI. These significant associations remained after further adjustment for cartilage volume, JSN and cartilage defects in the same compartments (Table 8.3).

### 8.3.5 Associations with knee pain

Figure 8.2 showed the associations between baseline OP phenotypes and increases in total WOMAC knee pain in different compartments. Established-OPs in total

tibiofemral and medial tibiofemoral compartment were positively associated with change in knee pain over 5 years, after adjustment for age, sex, BMI, BMLs and cartilage defects. In contrast, there was a significantly negative association between MRI-OPs in medial tibiofemoral compartment and change in total knee pain over 5 years, and this association remained significant after adjustment for age, sex, BMI, BMLs and cartilage defects in the same compartments (Table 8.4). MRI-OPs in total knee compartment were also negatively associated with knee pain change, but this did not reach statistical significance. All associations remained largely unchanged after further adjustment for NSAIDs usage and baseline WOMAC pain score (data not shown). No statistically significant associations were found for OPs in lateral tibiofemoral compartment (Table 8.4).

**Table 8. 4** Longitudinal associations of OP phenotype status and WOMAC knee pain changes in 5 years

| | **Total knee Pain** | |
|---|---|---|
| | Adjusted * β (95% CI) | Adjusted ** β (95% CI) |
| **OP phenotypes n=646** | | |
| ***Total tibiofemoral*** | | |
| No OPs (n=269) | *Ref.* | *Ref.* |
| MRI-OPs (n=315) | 0.21 (-0.82, 0.86) | -0.10 (-0.96, 0.76) |
| Established-OPs (n=62) | **2.52 (1.01, 4.03)** | **2.43 (0.84, 4.01)** |
| ***Medial tibiofemoral*** | | |
| No OPs (n=447) | *Ref.* | *Ref.* |
| MRI-OPs (n=155) | **-1.25 (-2.2, -0.30)** | **-1.51 (-2.50, -0.52)** |
| Established-OPs (n=43) | **2.91 (1.21, 4.60)** | **2.54 (0.74, 4.35)** |
| ***Lateral tibiofemoral*** | | |
| No OPs (n=287) | *Ref.* | *Ref.* |
| MRI-OPs (n=332) | 0.12 (-0.70, 0.94) | -0.05 (-0.91, 0.81) |
| Established-OPs (n=27) | 1.08 (-1.11, 3.27) | 0.35 (-1.95, 2.66) |

OP: osteophytes; Significant differences are shown in bold. Results of this table are generated from a linear regression model. * Adjusted for age, sex and BMI, ** Further adjusted for BMLs and cartilage defects;

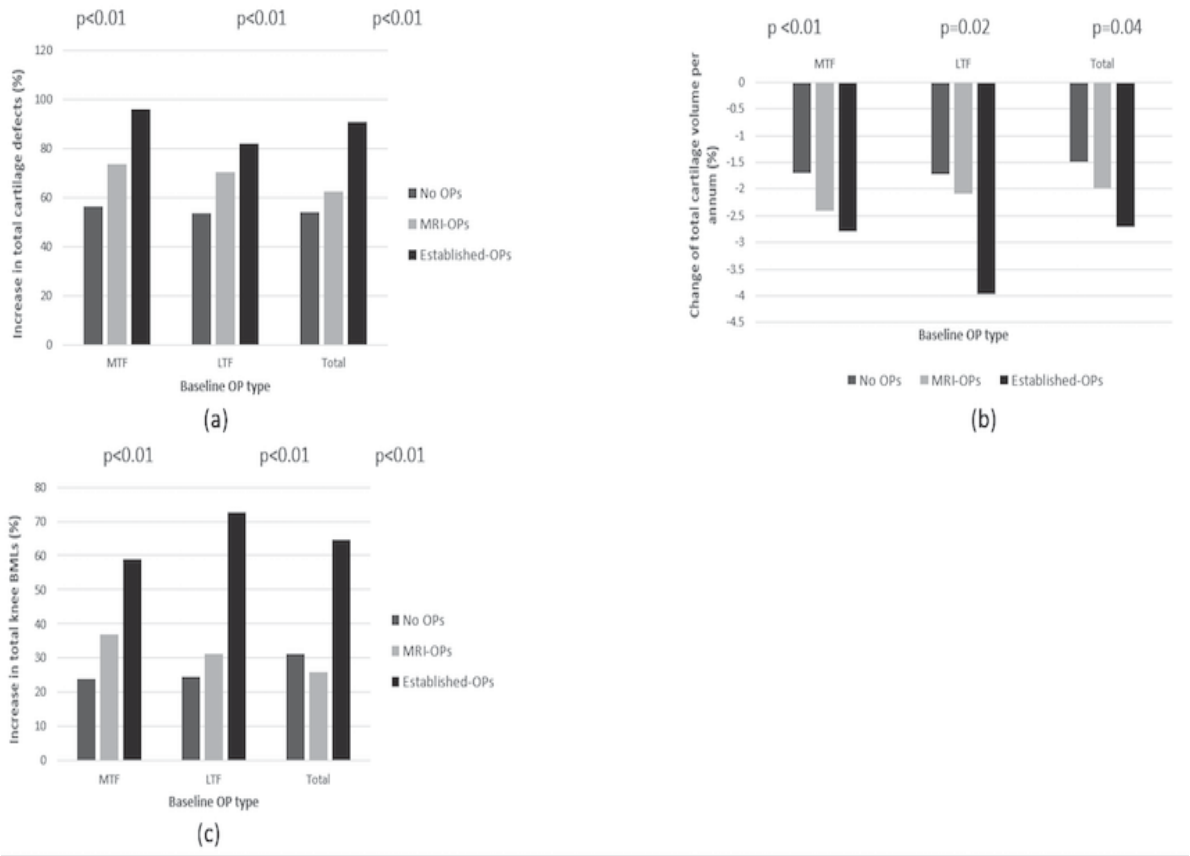



**Figure 8. 1** Associations of baseline osteophytes phenotypes with increases in total tibiofemoral

cartilage defects (a), change in cartilage volume (b), and increases in BMLs (c). OP: osteophytes; Total: total tibiofemral; MTF: medial tibiofemoral; LTF: lateral tibiofemoral.

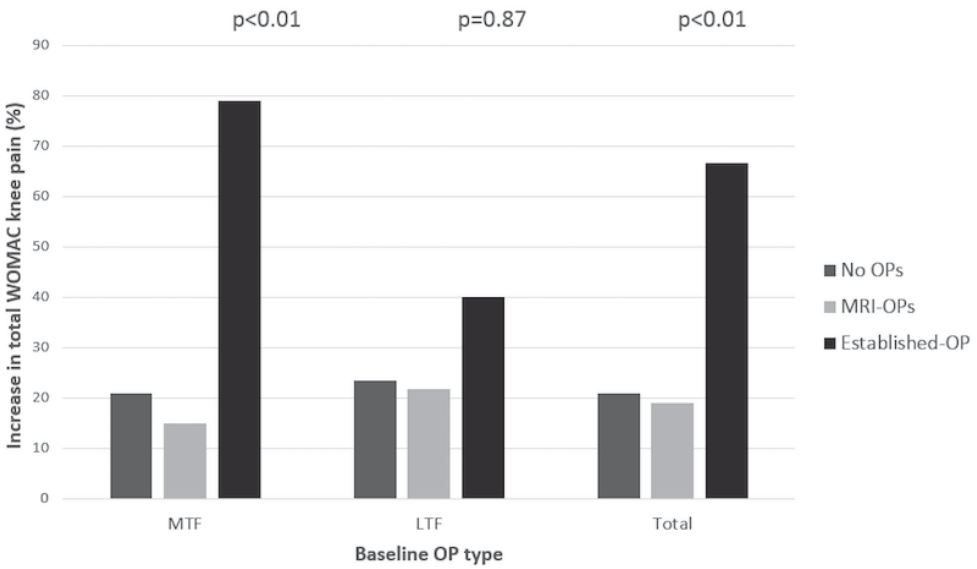



**Figure 8. 2** Associations of baseline osteophytes phenotypes with increases in total WOMAC knee pain

OP: osteophytes; Total: total tibiofemoral; MTF: medial tibiofemoral; LTF: lateral tibiofemoral.

## 8.4 Discussion

In this population-based cohort study, MRI-OPs at tibiofemoral compartment were prevalent, affecting 50% of older adults; in contrast, the prevalence of established-OPs was only 10%. Only 0.2% (2 case in this sample) had x-ray only OPs. Both categories of OP predicted progression of knee structural abnormalities in a dose-response manner, and the associations for MRI-OPs were not as prominent as those for established-OPs. This suggests MRI-OPs, which largely represent early OP formation, can also serve as a biomarker to predict knee structural progression over time.

Our current study confirmed that MRI-OPs were prevalent in an older population-based sample which highlights the need for an understanding of clinical relevance of these common findings. OPs are considered to be the hallmark of knee OA [6] and their size and extent are used for defining OA [30]. Despite the development and widespread use

of MRI in recent decades, conventional radiography remains the most commonly used imaging tool to detect OPs in research and clinical practice [103, 263]. The discrepancies of using MRI and radiography in detecting OPs have been reported previously [276]. MRI-defined OPs were present in 60% of older persons without radiographic OA [110], and were the most common abnormality that was found in 74% of all participants without radiographic evidence of OA [107].

Our study found that MRI-OPs and established-OPs are associated with knee structural changes in a dose response manner. Cross-sectional studies suggested that greater size of MRI-detected OPs correlated with higher Kellgren-Lawrence score, and increasing size and presence of MRI-defined OPs were associated with severity of knee OA [63, 111]. Another study reported that patients with central OPs detected by MRI had higher likelihood of full thickness or near-full thickness cartilage defects than patients without central OPs [188]. To the best of our knowledge, there are only two longitudinal studies examining the associations of MRI-detected OPs with knee structural changes so far. While one did not find any significant associations between MRI-detected OPs and knee structural progression [110], another reported that MRI-detected OP was an important factor in determining future total knee arthroplasty [277]. Our findings from the current longitudinal study were consistent, with OPs detected only by MRI but not by X-ray (MRI-OPs) being associated with increases in cartilage defects/loss and subchondral bone abnormalities over time. Our results are largely in line with findings from a previous case-control study which reported that hidden OPs on plain x-ray at femoral inter-condylar notch were at risk for the development of radiographic OA after 48 months [104], indicating MRI-OPs can serve as a biomarker for knee osteoarthritic structural progression before radiographic changes become evident.

Although knee OPs are associated with pain and predict pain weakly but more accurately than joint space narrowing, the longitudinal associations are inconsistent [44, 267, 278, 279]. In one prior study, increasing x-ray-detected OP size at baseline was reported to be associated with increasing WOMAC pain severity score [110]. In contrast, Link et al [111] reported that MRI-defined OPs were not associated with clinical findings as assessed with the WOMAC scores in patients with varying degree of OA. Neogi et al estimated the relationship of radiographic features with knee pain

and found that JSN was more strongly associated with knee pain than OPs [280]. A recent systematic review concluded that there was a lack of evidence on the association between OPs and knee pain [82], and it is still debatable if OPs are detrimental or beneficial for pain [97, 279]. Our data showed that while OPs detected only by MRI predicted a decrease of WOMAC knee pain over 5 years, established-OPs (both on MRI and x-ray) predicted an increase in knee pain over time. This is unexpected but the clinical relevance of MRI-OPs on knee pain progression remains uncertain. A previous study reported that removal of OPs from the arthritic compartment significantly increased the varus-valgus motion [98]. OPs have been considered an adaptive reaction of the joint to cope with instability and may play a compensatory role in the redistribution of forces to provide articular cartilage protection [97]. However, our data do not support this as both categories of OP were associated with worse structural change.

We employed a combination of WORMS and KOSS for the measurement of OPs. WORMS and KOSS scoring systems are two validated instruments which have good reliability to assess OPs semi-quantitatively on MR imagines [125, 196]. In our study, WORMS was used to divide the whole knee into 14 different subregions as it has one of the most complex differentiation of OP in terms of number of locations, and KOSS was used to score OP at each site. The reason for making this choice is because WORMS grading system has advantage of subdividing whole knee into different subregions which includes both marginal and central OPs, but its OP grading scale is more subjective . On the other hand, KOSS grading system has the advantage of quantitative OP grading scale for each subregion. The reliability of our measures were excellent.

There were several potential limitations in our study. One limitation is lack of skyline view to assess patellofemoral radiographic OPs, so we are unable to comment on the associations of patellofemoral MIR-OPs with OA progression. The patellofemoral joint is a common site of knee pain and contribute to functional limitation among OA patients [207, 208]. Future study are needed to investigate whether MRI-OPs in patellofemoral compartment have similar relationships with knee pain change as those in tibiofemoral knee compartments. Second, using higher field strength magnet than

1.5 T might be marginally more sensitive in detecting OPs; however, as reported previously [65], the results would not be markedly different as this benefit is modest. Third, follow-up MRI scans were only available in 395 out of 837 participants; However, there were no significant differences in demographic factors, ROA, baseline cartilage volume, defects and BMLs between the current study sample and the rest of cohort (data not shown). Last, the WOMAC knee pain questionnaire was not asked specifically for the right knee, while MRI scans were taken at right knee. Thus, the associations found between MRI-OPs and WOMAC knee pain change needs to be interpreted with caution.

In conclusion, MRI-OPs were associated with changes in knee structures, and the associations were similar but not as prominent as those for established-OPs. These suggest MRI-OPs may have a role to play in knee early-stage osteoarthritic progression.

# Chapter 9

# Summary and future directions

## 9.1 Summary

OA is one of the leading cause of pain, loss of function and disability amongst the elderly. The socioeconomic burden of OA is expected to increase dramatically in the coming decades as our society is increasingly aged and obese. Knees are the most common joint affected by OA [40]. Despite the high prevalence and heavy socioeconomic burden, the aetiology of OA remains unclear. Subchondral bone abnormalities have been recognized as a hallmark of knee OA, and studies have suggested that subchondral bone abnormalities may precede cartilage damage. This thesis has focused on two common subchondral bone abnormalities in knee OA: BMLs and MRI-detected OPs, and investigated relationships between cartilage morphology, systemic inflammation and knee BMLs as well as examining the predictive value of MRI-detected OPs on knee OA structural and symptomatic changes.

Chapter 4 was the first study to describe the natural history of PFJ BMLs. It also investigated longitudinal associations between PFJ BMLs changes, knee pain and knee cartilage morphology in older adults over 2.6 years. In this population-based sample of community-dwelling older adults, 27% had PFJ BMLs at baseline. Of these participants who had PFJ BMLs at baseline, PFJ BML scores increased in 24%, persisted in 44%, decreased in 32% and resolved completely in 21% of study participants. Incidence of PFJ BMLs in participants without PFJ BMLs at baseline was quite high over 2.6 years (26%). This suggestes that PFJ BMLs are not static. Change in PFJ BML score over 2.6 years was associated with changes in total WOMAC pain and pain when going up and down stairs over 5 years. These significant associations remained after adjustment for age, sex, BMI, smoking status, ROA, baseline PFJ BMLs and baseline cartilage defects. Participants with higher patellar cartilage volume at baseline had reduced risks of having an increase in PFJ BML score, and greater number of patellar cartilage defects at baseline were associated with increased risks of progressing PFJ BMLs; these associations were independent to structural abnormalities. The study suggests that changes in PFJ BMLs are clinically relevant, and PFJ cartilage morphology predicts increases in PFJ BMLs.

OA has traditionally been recognised as a non-inflammatory form of arthritis, driven by progressive deterioration of the entire joint [223]. However, increased numbers of studies have suggested that systemic inflammation is involved in its pathogenesis, albeit at lower levels than the established inflammatory arthropathies [154]. Chapter 5 described the cross-sectional and longitudinal associations between serum high sensitivity hs-CRP, BMLs and knee pain among patients with knee OA. Baseline serum levels of hs-CRP were associated with the presence of knee BMLs and total knee pain score in multivariable analyses. In longitudinal analyses, higher baseline hs-CRP level predicted increased BML score. In regard to knee pain, change in hs-CRP was positively associated with change in total knee pain score, but this was not independent of changes in BML score. These findings show that serum levels of hs-CRP are related to knee BML scores and knee pain both cross-sectionally and longitudinally, suggesting systemic inflammation is involved in the pathogenesis of BMLs and BML-associated pain. This adds the growing consensus that the development of OA is partly driven by low-grade systemic inflammation, but the underlying mechanisms are yet to be clarified.

Chapter 6 investigated the cross-sectional and longitudinal relationships between knee BMLs and IL-6/IL-23/IL-17 axis in patients with symptomatic knee OA. Baseline IL-6 (quartiles) were significantly related to total knee BMLs as well as an increase in BML score, independent to serum vitamin D levels. This is in line with our previous report [142] in which a higher circulating level of IL-6 independently predicted worsening knee pain. Baseline Il-17F and IL-23 (highest quartile vs others) was associated with increased BML score in females, but not in males. Combined with the associations that we found between IL-6 and BMLs, these results suggest that IL-6/IL-23/IL-17 axis may play a role in the aetiology and progression of knee OA, at least in females.

Although MRI can assess OPs in sites that are not easily visualised by conventional radiography [105], and at greater sensitivity than conventional radiographs for early detection of OPs [104], longitudinal studies about the predictability of MRI-detected OPs are rare. Chapter 7 described the prevalence of MRI-detected OPs in older adults and evaluated longitudinal associations with knee structural abnormalities as well as

knee pain. 85% of participants had MRI-detected OPs at baseline, while only 10% had radiographically-detected OPs. Longitudinally, baseline MRI-detected OPs predicted increased cartilage defects and BMLs, and loss of cartilage volume in a site-specific manner in multivariable analyses. Medial tibiofemoral and total OP scores were dose-dependently associated with total knee pain change over 2.6 and 5 years but these were not independent of baseline cartilage defects and BMLs. These indicate that MRI-detected knee OPs are common in older adults and may be an early marker of the disease process in knee OA.

Chapter 8 described the prevalence of OPs that can be detected by MRI but not by X-ray (MRI-OPs for short) in older adults and investigated longitudinal associations with knee pain and structures. 837 participants (mean age 62 years, 50% female) who had both MRI and radiographic scans at baseline were included. The prevalence of MRI-OPs was 75% while the prevalence of established-OPs (OPs detected by both MRI and X-ray) was only 10% in total knee at baseline. This is largely consistent with previous reports [107, 110], indicating MRI-OPs are highly prevalent in generally older population. Compared with participants without any OPs, participants with MRI-OPs and/or established-OPs had greater cartilage volume loss, increased cartilage defects and BMLs over 2.6 year. Unexpectedly, presence of medial tibiofemoral MRI-OPs predicted decreases in knee pain over 5 years, while established-OPs were associated with increased total knee pain in multivariable analyses. These findings show that MRI-OPs predict knee structural changes in a dose response manner. Unexpectedly, they predict lessening of knee pain over time, but the clinical relevance of this remains uncertain at this time.

In conclusion, this series of studies indicate that subchondral bone abnormalities such as BMLs and OPs are playing pivotal roles in the pathogenesis of knee OA. More importantly, systematic inflammation may be involved in the aetiology of BMLs in knee OA patients, particularly in women. MRI-detected OPs are common in older adults and can predict knee structural changes. Persons with knees that have OPs visiable on MRI but not radiographs may also experience knee osteoarthritic structural progression over time. Some recommendations for the future direction based on projects in this thesis are provided in the following section.

## 9.2 Future directions

This thesis presents several novel findings using participants from an population-based observational study of older adults, and a randomised controlled clinical trial of participants with symptomatic knee OA. The studies investigated the important roles of MRI-detected subchondral bone abnormalities in knee OA.

Chapter 4 was the first population-based longitudinal study that describes the natural history of PFJ BMLs. This study indicated a site-specific effect of cartilage morphology on PFJ BMLs. Additionally, the majority of the participants (87%) whose BMLs resolved had grade 1 PFJ BMLs, suggesting that mild PFJ BMLs have the potential to regress. Future work could further investigate which biological factors contribute to development and regression of BMLs and recommend therapies for those at risk. These factors could include physical activity, diet, genetic factors, knee alignment, medications, body weight, occupational factors, traumatic history, etc. The PFJ compartment is a common sourse of pain and functional limitation in OA patients [208], therefore, it is very important to fully investigate PFJ sources of pain and understand the underlying aetiology [207]. Future studies should focus on this particular compartment in order to better understand its role in knee OA.

Inflammation is now strongly implicated in the development and progression of OA [26, 142]. Chapter 5 investigated the relationships between serum levels of hs-CRP and knee BMLs as well as knee pain amongst knee OA patients. The findings suggest that higher serum levels of hs-CRP may reflect the inflammatory process in BMLs which in turn can be an underlying mechanism of knee pain. However, mechanism investigation is something that beyond the capacity of the current study, but is an avenus of furture research.

Chapter 5 was a secondary analysis conducted on a RCT; thus the findings may only be generalizable to patients with symptomatic knee OA but not general population due to the inclusion and exclusion criteria of the RCT. Moreover, the follow-up interval of 2 years may be insufficient to examine associations of serum levels of hs-CRP with knee pain. Observational studies suggest that associations may take 5 years to manifest

[225, 240]. Therefore, future investigations could benefit from studies including wider range of study participants with longer period of follow-up. If the link between hs-CRP, knee pain and knee BMLs are further confirmed by epidemiological studies, randomised controlled clinical trials targeting low-grade inflammation will be required to determine the effects of anti-inflammatory drugs on knee OA.

A number of investigations demonstrate roles of Th17 cytokines in the aetiology and progression of rheumatoid arthritis (RA) [256], a disease characterised by high levels of inflammation. OA is known to involve low level inflammation; experimental studies reporte that BMLs may originally correspond to an acute inflammatory response to cartilage breakdown products [87, 247]. Thus, it is rational to hypothesize that the Th17 cytokine family plays a role in the formation and progression of BMLs. Investigations in chapter 6 were the first study to assess longitudinal associations between IL-6, IL-23 and IL-17, and knee BMLs amongst knee OA patients. The data showed that higher serum IL-6 was associated with greater risk of an increase in knee BMLs over 2 years, and high levels of IL-17F and IL-23 predicted an increase in knee BMLs in females but not in males. This study demonstrated that OA is an inflammatory disease, and that inflammation is important early in the disase process, even before cartilage degeneration is visible [18]. Since there is a huge disability burden among patients with OA, DMOADs at the early stage of OA seem to be promising. Our improved understanding of the inflammatory pathways incuding the onset and progression of OA could advance development of targeted therapies. The reasons for the discordant findings between men and women in this study are not clear. Therefore, further studies should investigate interactions between gender and inflammatory processes in progression of OA. Additionally, the association between IL-6/IL-23/IL-17 axis and OA progression are also to be validated by other cohorts, and further investigating the relationship between other OA-related features such as meniscal tears, ligamentous lesion, cartilage degeneration and effusion-synovitis and IL-6/IL-23/IL-17 axis will provide a more comprehensive understanding of inflammatory processes in OA. Once the role of IL-6/IL-23/IL-17 axis in OA progression is disentangled, further clinical and experimental studies can be conducted to screen out effective drugs aimed at interference this axis and thereby to modify the development of OA.

Conventional radiographs are the current standard for establishing a radiographic diagnosis of knee OA [30, 281]. Hallmarks of OA include the presence of marginal OPs and joint space narrowing [6, 262]. However, associations between radiographic abnormalities and clinical features of OA are poor [282]. Despite the widespread use of MRI depicted structural abnormalities of OA, longitudinal studies looking at MRI-detected OPs and OA clinical features are rare. Chapter 7 demonstrates that MRI has much higher sensitivity than did conventional radiography for detection of OPs. MRI-detected OPs are associated with knee structural abnormalities both cross-sectionally and longitudinally. MRI-detected OP could be a very useful clinical tool to monitor treatment response once MRI-detected OP is confirmed as an important OA biomarker in other epidemiological studies. Since the natural history of MRI-detected OPs has not yet been described, future studies can investigate the natural history of MRI-detected OPs and to examine whether MRI-detected "early" OPs are important in terms of OA structural and symptomatic progression. Dose-response associations between MRI-detected OPs and WOMAC knee pain were observed but these were largely dependent of knee structural abnormalities. Oveall, these results suggest that MRI-detected OPs may be an "early" marker of the disease process in knee OA; thus "early" OP formation may be an useful predictor of disease progression.

Previous studies indicate that OP formation may precede cartilage damage [104, 276], but longitudinal studies are needed to confirm whether radiographically normal knees with MRI-detected OPs can induce cartilage damage and exacerbation of pain. Chapter 8 showed the prevalence of OPs visiable by MRI but not by standard X-ray (MRI-OPs) was much higher (75% vs 10%) than the prevalence of established-OPs (OPs detected by both MRI and X-ray) as expected. This is largely consistent with other studies [107, 276]. This study focused on "early OA" population, which has not been well defined. The definition of "early OA" using MRI as a imaging tool besides clinical characteristics remains to be developed and validated. This study provides evidence that MRI-OPs can serve as a biomarker in identifying patients at a risk of osteoarthritic progression. Development and validation of a comprehensive early OA diagnosis system so that this group of patients can be identified earlier and more effectively is an essential goal for future work. Total knee arthroplasty (TKA) is an important outcome in end-stage OA, both from a research and a clinical perspective. Prevention or delaying

of TKA represents the ultimate clinical goal for any therapeutic attempts. It will be interesting to examine whether MRI-OPs can be used as a prognostic marker to predict TKA occurrence in long-term follow-up studies in the future.

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# APPENDICES

## Appendices 1-5 have been removed for copyright or proprietary reasons. They are the following published articles:

Zhu Z, Ding C, Jin X, Antony B, Han W, Laslett LL, Cicuttini F, Jones G. Patellofemoral bone marrow lesions: natural history and associations with pain and structure. Arthritis Care & Research. 2016 Nov; 68(11):1647-1654.

Zhu Z, Jin X, Wang B, Wluka A, Antony B, Laslett LL, Winzenberg T, Cicuttini F, Jones G, Ding C. Cross-sectional and longitudinal association between serum levels of high-sensitivity c-reactive protein, knee bone marrow lesions, and knee pain in patients with knee osteoarthritis. Arthritis Care & Research. 2016 Oct; 68(10):1471-7.

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Zhu Z, Laslett LL, Jin X, Han W, Antony B, Wang X, Lu M, Cicuttini F, Jones G, Ding C. Association between MRI-detected osteophytes and changes in knee structures and pain in older adults: a cohort study. Osteoarthritis Cartilage. 2017 Jul; 25(7):1084-1092.

Zhu Z, Laslett LL, Han W, Antony B, Jin X, Lu M, Cicuttini F, Jones G, Ding C. Associations between MRI-detected early osteophytes and knee structure in older adults: a population-based cohort study. Osteoarthritis Cartilage. 2017 Dec; 25(12):2055-2062.

## WOMAC Questionnaires _VIDEO Study

**Site Number:** □ **Randomisation Number:** □□□□

**Subject Initials:** □□□ **Study Selected Knee** □□□□□

2 Rate the following today. Place a mark on the line:

***3 Referring to your knee, Do you have (This is not in questionnaire but is implicit)***

**PAIN** none severe

Walking on a flat surface

Going Up and down stairs

At night while in bed

Sitting or lying

Standing upright

**STIFFNESS**

After first awakening

Later in the day

**4 FUNCTIONAL DEFICIT**

Descending stairs

Ascending stairs

Rising from bed

Rising from sitting

Putting on socks

Taking off socks

Bending to the floor

Lying in bed

Walking on flat

Getting in/out of bath

5 Standing

Sitting

Getting in/out of the car

6 Getting on/off toilet

Heavy domestic chores

Light domestic chores

Shopping

Menzies Research Institute Tasmania

*Appendix B. WOMAC Questionnaires*

TASOAC

ID Number ☐☐☐☐

# TASOAC
# Phase 4 Questionnaire

Prof. Graeme Jones

## IDENTIFYING DATA

Date Phase 1 Questionnaire Completed: ☐☐ / ☐☐ / ☐☐☐☐

Date Phase 2 Questionnaire Completed: ☐☐ / ☐☐ / ☐☐☐☐

Date Phase 3 Questionnaire Completed: ☐☐ / ☐☐ / ☐☐☐☐

Date Phase 4 Questionnaire Completed: ☐☐ / ☐☐ / ☐☐☐☐

**Instructions for completing the questionnaire:**

*All questionnaires will be submitted electronically. By following these instructions you will be assisting with this process.*

Please answer all questions to the best of your ability (leave blank if unknown).
Do not put lines through irrelevant questions as it upsets the scanning machine.

Write in **BLOCK LETTERS** using the boxes where provided

Use a blue pen

Cross out any mistake & write correct answer just below the relevant box

Indicate your response by filling in the circle next to the most appropriate answer or by writing clearly in the box or space provided.

Your answers will be completely confidential.

Please notate any queries and they will be reviewed at your appointment.

Example:

Shade Circles Like This--> ●

Not Like This--> ⊗ ✓

## 4.7 Rate the following today for your RIGHT knee

(if your **RIGHT** knee has been replaced rate your left knee)



This section assesses pain, stiffness and functional deficit on a scale from 1 - 10

| Example | none | | | | | | | | | severe |
|---|---|---|---|---|---|---|---|---|---|---|
| Example of no pain | ● 1 | ○ 2 | ○ 3 | ○ 4 | ○ 5 | ○ 6 | ○ 7 | ○ 8 | ○ 9 | ○ 10 |
| Example of severe pain | ○ 1 | ○ 2 | ○ 3 | ○ 4 | ○ 5 | ○ 6 | ○ 7 | ○ 8 | ○ 9 | ● 10 |

**1. Referring to your knee only how much pain do you experience**

| | none | | | | | | | | | severe |
|---|---|---|---|---|---|---|---|---|---|---|
| a. Walking on a flat surface | ○ 1 | ○ 2 | ○ 3 | ○ 4 | ○ 5 | ○ 6 | ○ 7 | ○ 8 | ○ 9 | ○ 10 |
| b. Going up and down stairs | ○ 1 | ○ 2 | ○ 3 | ○ 4 | ○ 5 | ○ 6 | ○ 7 | ○ 8 | ○ 9 | ○ 10 |
| c. At night while in bed | ○ 1 | ○ 2 | ○ 3 | ○ 4 | ○ 5 | ○ 6 | ○ 7 | ○ 8 | ○ 9 | ○ 10 |
| d. Sitting or lying | ○ 1 | ○ 2 | ○ 3 | ○ 4 | ○ 5 | ○ 6 | ○ 7 | ○ 8 | ○ 9 | ○ 10 |
| e. Standing upright | ○ 1 | ○ 2 | ○ 3 | ○ 4 | ○ 5 | ○ 6 | ○ 7 | ○ 8 | ○ 9 | ○ 10 |

**2. Referring to your knee only how much stiffness do you experience**

| | none | | | | | | | | | severe |
|---|---|---|---|---|---|---|---|---|---|---|
| a. After first awakening | ○ 1 | ○ 2 | ○ 3 | ○ 4 | ○ 5 | ○ 6 | ○ 7 | ○ 8 | ○ 9 | ○ 10 |
| b. Later in the day | ○ 1 | ○ 2 | ○ 3 | ○ 4 | ○ 5 | ○ 6 | ○ 7 | ○ 8 | ○ 9 | ○ 10 |

**3. Referring to your knee only how much functional deficit do you experience when**

| | none | | | | | | | | | severe |
|---|---|---|---|---|---|---|---|---|---|---|
| a. Descending stairs | ○ 1 | ○ 2 | ○ 3 | ○ 4 | ○ 5 | ○ 6 | ○ 7 | ○ 8 | ○ 9 | ○ 10 |
| b. Ascending stairs | ○ 1 | ○ 2 | ○ 3 | ○ 4 | ○ 5 | ○ 6 | ○ 7 | ○ 8 | ○ 9 | ○ 10 |
| c. Rising from bed | ○ 1 | ○ 2 | ○ 3 | ○ 4 | ○ 5 | ○ 6 | ○ 7 | ○ 8 | ○ 9 | ○ 10 |
| d. Rising from sitting | ○ 1 | ○ 2 | ○ 3 | ○ 4 | ○ 5 | ○ 6 | ○ 7 | ○ 8 | ○ 9 | ○ 10 |
| e. Putting on socks | ○ 1 | ○ 2 | ○ 3 | ○ 4 | ○ 5 | ○ 6 | ○ 7 | ○ 8 | ○ 9 | ○ 10 |
| f. Taking off socks | ○ 1 | ○ 2 | ○ 3 | ○ 4 | ○ 5 | ○ 6 | ○ 7 | ○ 8 | ○ 9 | ○ 10 |
| g. Bending to the floor | ○ 1 | ○ 2 | ○ 3 | ○ 4 | ○ 5 | ○ 6 | ○ 7 | ○ 8 | ○ 9 | ○ 10 |
| h. Lying in bed | ○ 1 | ○ 2 | ○ 3 | ○ 4 | ○ 5 | ○ 6 | ○ 7 | ○ 8 | ○ 9 | ○ 10 |

| **4.7 Question 3 continued** | none | | | | | | | | | severe |
|---|---|---|---|---|---|---|---|---|---|---|
| i. Walking on flat surface | ○ 1 | ○ 2 | ○ 3 | ○ 4 | ○ 5 | ○ 6 | ○ 7 | ○ 8 | ○ 9 | ○ 10 |
| j. Getting in/out of the bath | ○ 1 | ○ 2 | ○ 3 | ○ 4 | ○ 5 | ○ 6 | ○ 7 | ○ 8 | ○ 9 | ○ 10 |
| k. Standing | ○ 1 | ○ 2 | ○ 3 | ○ 4 | ○ 5 | ○ 6 | ○ 7 | ○ 8 | ○ 9 | ○ 10 |
| l. Sitting | ○ 1 | ○ 2 | ○ 3 | ○ 4 | ○ 5 | ○ 6 | ○ 7 | ○ 8 | ○ 9 | ○ 10 |
| m. Getting in/out of the car | ○ 1 | ○ 2 | ○ 3 | ○ 4 | ○ 5 | ○ 6 | ○ 7 | ○ 8 | ○ 9 | ○ 10 |
| n. Getting on/off the toilet | ○ 1 | ○ 2 | ○ 3 | ○ 4 | ○ 5 | ○ 6 | ○ 7 | ○ 8 | ○ 9 | ○ 10 |
| o. Heavy domestic chores | ○ 1 | ○ 2 | ○ 3 | ○ 4 | ○ 5 | ○ 6 | ○ 7 | ○ 8 | ○ 9 | ○ 10 |
| p. Light domestic chores | ○ 1 | ○ 2 | ○ 3 | ○ 4 | ○ 5 | ○ 6 | ○ 7 | ○ 8 | ○ 9 | ○ 10 |
| q. Shopping | ○ 1 | ○ 2 | ○ 3 | ○ 4 | ○ 5 | ○ 6 | ○ 7 | ○ 8 | ○ 9 | ○ 10 |